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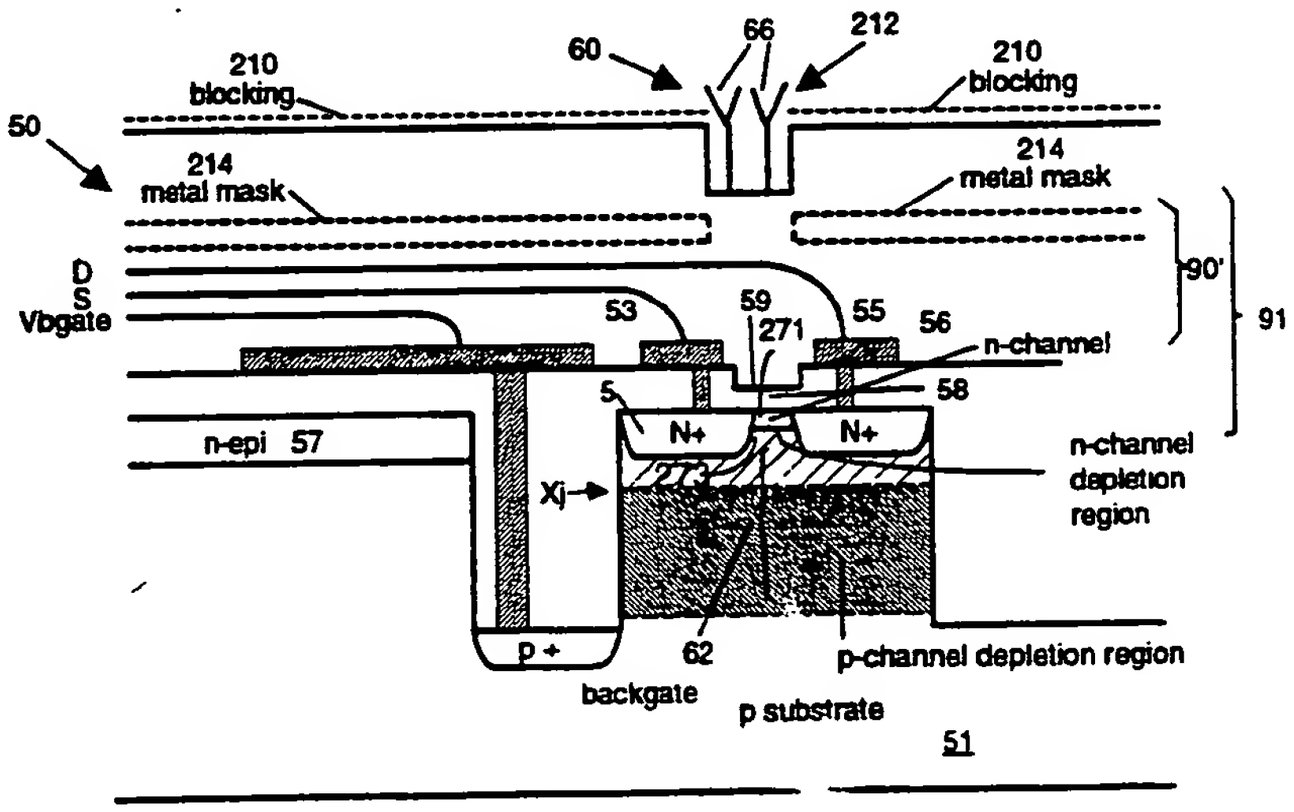

(57) Abstract

A method and application for detecting and measuring the presence of a binding target material (64) employs a semiconductor device (50) having a receptor-covered surface (90) topgate (60), separated by a dielectric layer (58) from a substrate (56). Receptors (66) attached to this surface exhibit a chemical selectivity function. Binding occurs in a test solution (52), with charge associated with the target material (64) modulating at least one device characteristic. According to the present invention, measurement may occur under dry conditions, at a time and location different from when binding occurred, thus substantially eliminating problems associated with ionic shielding and reference electrodes, so prevalent with prior art wet measurement techniques. Preferably the device (50) includes backgate (62) to which a bias may be applied to restore the device's pre-binding characteristics. Measurement of the restorative backgate bias provides a signal indicating binding of the desired target material.

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METHOD AND DEVICE FOR BIOCHEMICAL SENSING

RELATIONSHIP TO EARLIER FILED APPLICATION

This application claims priority from United States patent application serial number 07/781,479, filed on October 21, 1991.

FIELD OF THE INVENTION

This invention relates to sensing and measuring chemicals in general, and more particularly to methods and apparatus for sensing and measuring chemicals, biochemicals, molecules and sub-molecular components including ions, using semiconductor sensors.

BACKGROUND OF THE INVENTION

Semiconductor sensors for detecting biochemical reactions are known in the art, as exemplified by U.S. patent no. 4,180,771 to Guckel (1979). Figure 1 depicts a typical such prior art sensor 2 used to measure the attachment to a solid substrate surface 4 of a desired chemical compound 6 in a solution 8.

Sensor 2 typically is fabricated like a metal oxide silicon ("MOS") field effect transistor, wherein region 10 functions like a channel between source and drain regions 12, 14, and region 16 functions like a gate, but without metalization. Using receptor-type mechanisms 18, region 4 is made sensitive to (and encourages adhesion or attraction with) a desired target substance 6. Alternatively, receptor-like mechanisms 18 may be attached to the device gate 16.

Although region 4 should be relatively insensitive and non-reactive to other chemicals, such as 20, but in practice region 4 can respond non-specifically and attach with other than desired target substance 6. For example, solution 8 may also include charged particles 22 of varying size, including positive and negative ions. In biochemical sensing applications, a suitable biochemical environment for the receptor 18 and bio-target 6 dictates that test solution 8 have a relatively large ionic concentration. Unfortunately, relatively large ion concentration for the test solution 8 can adversely affect biochemical measurement due to ionic shielding.

Prior art measurements use a reference electrode to obtain stable and reproducible measurements, which electrode may be attached to sensor 2, e.g., electrode 24, or not attached, e.g., electrode 24' (shown in phantom). The reference electrode is coupled to a reference potential V_r, (e.g., ground) and completes an electrical circuit, apparently to provide proper sensor biasing and to eliminate drift. Various bias potentials V_{sr}, V_{gr} and V_{dr} are coupled to the sensor, typically referenced to V_r. One or more measuring devices, indicated generically by 26, are also coupled to the sensor 2.

If the target substance 6 is present in solution 8, it should attach or bind to receptor 18, bringing electrical charges associated with the target substance. Target 6 attachment also brings mass to receptor 18, and can alter receptor 18's contact potential as well.

Thus, during binding or attachment, these electrical charges associated with receptor 18 influence charge present at region 4 (or gate 16, alternatively) and can measurably alter device 2's substrate bias, which can affect device 2's operating characteristics, including conductance and threshold voltage. By monitoring sensor 2 with detection and measurement equipment 26, these characteristic changes may be detected, indicating a binding of the target substance 6.

Further, charges at region 4 can also manifest a contact potential that tends to vary somewhat logarithmically with the charge concentration, a phenomenon sometimes used in sensing pH. It is characteristic of the prior art that measurements are made when binding of the target substance occurs, e.g., while sensor 2 is still immersed in solution 8.

Unfortunately such prior art sensors and sensing techniques have several deficiencies, including the use of reference electrodes, the inability to meaningfully directly measure charged particles including biochemicals (especially where the test solution is rich in ions), relative device insensitivity and drift, relatively high sensor production cost, and the perceived necessity to make "wet" measurements, i.e., while the sensor is in solution.

Prior art device reference electrode 24 or 24' unfortunately can contaminate the solution 8, and corrupt measurements. Further, the reference electrode bias V_r can interact unfavorably with any ions 22 present in the solution, resulting in ionic charge separation and polarization. Because even minute movement or agitation of solution 8 circulates these ions, potential disturbances are created that can affect measurement accuracy.

Further, sensing devices and procedures such as depicted in Figure 1 do not provide meaningful detection and direct measurement of charged particles, especially such particles exceeding a few angstrom in size, where the test solution has high ion concentration. In some

applications, the target to be detected is a charged particle 28 that may be several tens of angstroms or greater in size.

Unfortunately in Figure 1, ions 22, 30 in solution 8 can screen out and thus mask or shield the target charged particles. Thus, charges associated with the receptors and/or targets may be neutralized (in whole or part), thus masking the desired attachment signal.

To better appreciate the adverse effects of ionic shielding, assume that receptor 18 in Figure 1 has been charged positively (e.g., as a result of pH buffering of the solution 8), and that target material 6 is not yet introduced into the solution. Since solution 8 may includes ions 28, 30 of either polarity, mobile negative ions (assume 30) are attracted to receptor 18, and mobile positive ions (assume 28) are repelled. The polarized negative ions 30 shield or nullify the receptor 18 charge, causing a net charge of zero to be seen somewhat below the substrate surface 4. At the interface between the receptors 18 and substrate surface 4 the electric field is substantially zero, and thus the underlying FET is not influenced.

When added to the solution, target material 6 binds selectively to the mating receptor 18. But any material 6 charge experiences shielding due to ions in the solution, and produces near zero charge somewhat below the substrate surface 4, as indicated by the associated electric field. Thus, although a charged target material 6 has bound to the receptor 18, shielding prevents meaningful detection by device 2. Device 2's failure to sense attachment is a chronic problem with prior art devices, and may result in a false negative report. But to support certain medical and biochemical reactions of interest (e.g., many antibody-antigen reactions), the solution must have a relatively high ionic concentration that can result in a shielding length substantially masking, reducing or interfering with detection of the binding-charge induced signal of interest.

This apparent resultant low sensitivity associated with prior art FET type sensors (e.g., sensor 2) has caused such devices to be disfavored as sensors for the direct detection in solution of charged molecules, especially biochemicals.

Prior art sensor insensitivity is especially troublesome where relatively small changes (ΔS) in a signal (S) are to be measured. Rather than being able to provide a direct measurement of ΔS, such prior art devices sense $\log(S + \Delta S)$ and provide a signal proportional to $\log(S + \Delta S) - \log(S)$, at best a relatively insensitive indirect measurement of ΔS. Logarithmic dependent measurements are believed to account for the low sensitivity of typical prior art pH sensors.

Ionic shielding is not the only disadvantage with prior art in-solution sensor measurements. Wet testing can subject the measurements to drift resulting, for example, from ion movement within the solution, and from reference electrode contamination.

Further, in a given application the measurement and detection equipment 26 may require sophisticated and expensive components. Under such circumstance, having to "wet test" requires that the test and detection/measurements occur essentially at the same time and place as the target binding. This restriction can preclude the use of sensors if sophisticated equipment is not readily available in the region where the testing (that possibly leads to binding) occurs.

It would be advantageous if after possible binding, the sensor could be sent, preferably dry, to a remote facility for detection and measurement of any target substance attachment using sophisticated equipment not available at the testing/binding region. Unfortunately, such "dry testing" is not practiced with prior art devices and procedures such as depicted in Figure 1.

Fabricating many prior art sensor devices is sufficiently expensive as to preclude "use once and discard" practice. Similarly, often the receptor material is scarce or very expensive. Clearly it would be advantageous if devices and/or their receptor materials could be used more than once. In addition, sufficiently inexpensively fabricated devices could be provided in arrays to permit simultaneous testing, included automated testing for multiple target materials simultaneously (e.g., multiple disease antigens), included automated testing.

Many prior art sensors have limited sensitivity, limited sensor gain, and/or device drift, unfortunate limitations since in many clinical applications, a target biological analyte may exist in a minute concentration, i.e., a few ng/ml for proteins in blood serum. Substantially more sensitive devices would permit the simultaneous use of several different dedicated receptors to provide more rapid (and thus less expensive) testing, including differential analysis testing.

In short, there is a need for an inexpensive immunosensor, preferably a IC-compatible (preferably integrated with signal enhancing, control and other environmental sensors, all on-chip), FET-type device that can be inexpensively mass produced using standard semiconductor fabrication technology. Such device should reliably measure biochemical information with high sensitivity, and be substantially free of signal drift.

Further such devices should include multiple receptors, some of which may be dedicated to binding different target materials, and should further include a mechanism for discerning which of several target materials have in fact bound. Further, there is a need for devices that may be fabricated and used in arrays, including arrays containing sensors with multiple types of

receptors. Such arrays would promote rapid and relatively inexpensive testing, including differential and confirmational analysis testing, including self-testing of the devices themselves, and testing that environmental feature such as temperature and pH are appropriate to insure that valid testing has occurred.

Preferably such device, and a methodology using such device, should not require a reference electrode, and should be capable of making measurements under wet or dry conditions. Further such device and method should enable detection of a contact potential resulting from the binding of a target material and a receptor, and should include mechanisms to eliminate false positive and false negative measurements resulting from non-unique pH_{zc} values.

Further, such device and methodology should provide mechanisms for enhancing the sensitivity of the device per se, for enhancing the effective amount of charge binding to the device, and for amplifying the signal detected by the device. Preferably such mechanisms should be usable and reusable under wet or dry measurement conditions.

Finally, such device and methodology should be useful in a wide spectrum of applications including biochemical sensing and measurement, DNA research, pH and hydrogen sensing, pollution sensing, optical and photodetector sensing, pyroelectric sensing, magnetic and force sensing including piezoelectric sensing.
The present invention provides such devices and methodologies.

SUMMARY OF THE INVENTION

In a first aspect, the present invention provides a field effect type device that can be used for wet or dry detection and measurement of a binding target material, without requiring a reference electrode. In a first embodiment, the device includes a semiconductor material whose upper surface is covered by an insulator layer supporting a top gate, and a lower surface that supports a bottom gate. When coupled to a power source, the device creates a field effect region, and includes a channel capable of conduction as a function of electrical activity at the top gate and/or bottom gate. The top gate preferably is covered by a binding layer that selectively provides a chemical reaction function in the presence of a predetermined class of target, such as charged particles, bio-particles, chemicals, etc. In a second embodiment, a distributed channel bipolar device having bipolar and MOS device characteristics is employed as a sensor.

Such devices may be implemented in a variety of ways using field effect device phenomena, such associated with a junction field effect transistor ("FET"), an exposed insulator FET ("EIFET"), a non-metalized gate metal-oxide-silicon field effect transistor ("MOSFET"), a non-metalized gate metal-insulator field effect transistor ("MISFET"), a heterojunction device, and

a field effect capacitor. Further, the field effect region of the device may be operated in a variety of modes, such as enhancement, depletion, inversion or accumulation. In the first embodiment, the bottom gate may be implemented in several ways, including as a PN junction, a PIN region, and an inversion channel/substrate. Preferably the bottom gate structure is relatively lightly doped such that a voltage applied thereto is dropped primarily away from the channel, to enhance sensitivity of the device.

In use, the device is exposed to a solution containing a target material with which the binding layer will attach. If attachment occurs, the resultant change in attachment charge and/or contact potential will alter the device's quiescent state. Such induced charge effects may be used with a FET-type sensor statically, sequentially, or transiently. Suitable monitoring equipment coupled to the device can detect this change, confirming that the target substance is indeed present, and providing an quantitative measurement. Various amplifying means and feedback features may be to enhance sensitivity and performance.

In a second aspect, the present invention provides measurement of the device to occur in a dry, or at least quasi-dry state. Measurements may also be made in the conventional wet state. In dry/quasi-dry testing, the device is exposed to a test solution wherein binding with a target substance may occur. The device is then dried such that attached or bound target substance remains attached to the device. The device is then measured dry (or re-wetted), at a later time and different location, if desired.

The present invention's ability to dry measure avoids the ionic shielding problems and resultant low sensitivity associated with prior art wet measurement techniques. As a result, the present invention permits meaningful direct measurements of charged matter, including particles, ions, many biochemicals, nucleic acid chains and components such as DNA and DNA segments, and so forth.

In further contrast to the prior art, the present invention requires no reference electrode, and thus avoids reference electrode contamination of the test solution, signal drift, and ionic disturbances. Mechanisms are disclosed for selectively attaching receptors to desired regions of the sensor, to enhance performance and to protect any other components on the substrate from deleterious attachment effects.

In yet another aspect, the present invention provides various mechanisms, suitable for wet or dry measurements, for enhancing the attachment of target material to the device, for enhancing sensitivity of the device to attached target material, for providing confirmational data including confirmation that the devices themselves are functioning, and for processing signals from the device representing attachment.

Other features and advantages of the invention will appear from the following figures and from the following description, wherein the preferred embodiments are set forth in detail.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 depicts a generalized sensor and sensor measurement, according to the prior art;

FIGURE 2A is a perspective depiction of an EIFET sensor, according to the present invention;

FIGURE 2B is a side view of an EIFET sensor, according to the present invention;

FIGURE 3A depicts a sensor and generalized sensor measurement according to a first embodiment of the present invention;

FIGURE 3B depicts a sensor and generalized sensor measurement according to a second embodiment of the present invention;

FIGURE 4 depicts an alternative embodiment for a sensor, using a distributed channel bipolar device;

FIGURE 5 depicts an embodiment for a sensor array that includes sensors having multiple receptors including neutral receptors, and sensors with on-chip memory;

FIGURE 6A depicts a cascode arrangement for signal enhancement, according to the present invention;

FIGURE 6B depicts an embodiment wherein device current is constant and back bias is modulated and sensed;

FIGURE 7A depicts sequential use of a blocking agent, according to the present invention;

FIGURE 7B depicts the use of sandwich-like structures and conglomerates to enhance detection, according to the present invention;

FIGURE 7C depicts the use of beads to enhance detection, according to the present invention;

FIGURE 7D depicts the use of conjugates to enhance detection, according to the present invention;

FIGURE 8A depicts changes in the characteristics of a device according to the present invention, in response to different attachment phenomena;

FIGURE 8B is a bar graph conductivity depiction of the slope of the data shown in FIGURE 7A;

FIGURE 8C is a bar graph depiction of conductivity change in a device according to the present invention, in response to exposing the device to various solutions including a denaturing solution.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Figures 2A and 2B depict a sensor 50 implemented as a semiconductor field effect device, such as an FET. Device 50 may be fabricated in a variety of configurations, including a junction FET ("JFET"), a metal-oxide-silicon FET ("MOSFET"), a metal-insulator-silicon FET ("MISFET"), an exposed insulator FET ("EIFET"), an ion sensitive FET ("ISFET"), a distributed channel bipolar device ("DCBD"), a heterojunction device and a capacitor. Further, semiconductor device 50 may be operated in enhancement mode, depletion mode, or inversion mode. It is significant to note that no reference electrode is depicted or required according to the present invention.

Device 50 includes a semiconductor substrate bulk 51 having a source 53, a drain 55, an FET channel 56 capable of electrical conduction, an insulation layer 58 such as SiO_2, Si_3N_4, etc., an exposed insulator topgate 60, and a bottom or lower gate 62. According to the present invention, the conductivity of channel 56 may be modulated by electrical signals and/or charge present at topgate 60 and/or bottom gate 62. Preferably device 50 is an exposed insulator FET, or "EIFET", with a relatively deep channel 56 (under no backgate bias) to increase device detection sensitivity.

The uppermost surface 59 of insulator layer 58 is preferably covered with a protective moisture blocking film 90 that is relatively inert chemically, impervious to any corrosive components in solution 52, will not contaminate the test solution, and provides an outer surface with many sites for binding with target specific receptor substances 66. Applicant has found parlyene to function especially well as film 90, although other materials could also be used. Further, this structure allows the topgate to float at the potential of the test solution, thus avoiding a topgate bias that could influence the binding reactions at the topgate and introduce erroneous signals. As a result, the surface of film 90 can be made selectively reactive without contamination risk.

Applicant's depletion mode EIFET device (Figures 2A and 2B) had a 92.5 nm thickness for layer 58, an approximately 100 nm parylene film layer 90, an n-epitaxial layer channel 56 with

4.6 microns thickness, doped at about 10^{15}/cc (calculated to be 3.4 microns absent back bias), with W/L ≈ 1432 and source-drain distance L = 20 microns. As shown by Figure 2A, the gate 60 was fabricated with a meanderline pattern to provide a large W/L using the laboratory equipment at hand, to thus enhance device sensitivity. In Figure 2A, backgate 62 is the pn junction between substrate 51 (p material) and channel 56 (n-epi region 57). Of course other polarity dopants could be used.

It was not feasible to fully deplete through the device channel due to leakage current generation problems associated with the prototype. However, even with partial depletion of the epi channel, large receptor and target attachment signals were observed. These signals would be even larger for the near total channel depletion condition, 1 μ drain-source dimensions (thus increasing W/L) that a commercially fabricated device should produce. A commercially fabricated device would further permit very high backgate amplification, with further sensitivity enhancement.

According to the present invention, attached charge influences an underlying channel region in a measurable fashion. It is therefore desired that receptors 66 be affixed to the present invention 50 over the topgate region 60, but not elsewhere. In a conventional integrated circuit, the overlying field oxide layer is of no use in protecting underlying regions against attached surface charge. With reference to Figure 2B, selective receptor attachment can occur in several ways. In a first method, the uppermost surface of device 50 is masked with an inert agent or blocking agent 210 that blocks attachment to material 66 such that only the region 212 over the topgate 60 remains unmasked and thus receptive. The device is then exposed to a solution containing the desired receptor material 66, which attaches over the topgate region 212, but not elsewhere due to the blocking material mask 210.

Alternatively, mask 210 may be a preselected blocking chemical with a predetermined charge polarity, chosen such that underlying regions (e.g., devices or circuits) are not unduly influenced by charge attached to mask surface 210. Where a specific masking chemical layer 210 has been patterned to protect underlying regions, post target attachment treatment can be used, such as preparation with a buffered pH solution corresponding to the layer 210's isoelectronic point.

In a second method, the device includes metalization patterns 214 that preferably shield all of the device save for the desired topgate region 212. Preferably the metal mask 214 is covered by a layer of a material 90, e.g. parylene, to minimize possible test solution contamination by the mask. The device is then exposed to a solution containing the desired receptor material 66. Receptors 66 attaching over the topgate region 212 will be in field communication with the underlying channel region 56, whereas receptors attaching elsewhere will have their charge

(and indeed any subsequently attached target material charge) shielded from influencing regions outside of the topgate region 212 by the metal mask 214, which is coupled to a DC potential or ground.

The potential is selected so underlying regions are not unduly influenced by charge carrying chemicals attached above the shield region 214. Thus, shielding, including buried metal masking, is especially desired where the substrate 51 includes other components, such as depicted in Figure 5. But for the desired shielding action provided by the mask 214, performance of other components (e.g., 100, 102, 104) disposed beneath receptor material 66 could undesirably be influenced by the resultant electric field changes produced by the receptors and/or any targets attached thereto.

A selected primary receptor 66 is selectively attached to the outer surface of film 90, during or after fabrication of device 50. Essentially primary receptor 66 dedicates device 50 to bindingly detecting a mating target substance 64, but not other substances 68, 70, 72 that may be present in the solution (see Figures 3A, 3B), which other substances may include charged materials and positive and negative ions. As depicted in Figures 5A and 5B, device 50 may in fact include two or more receptor types 66, 66', each of which is dedicated to bind with a different target material.

Alternatively, where simultaneous testing for multiple target materials is desired, an array of sensors incorporating one or more specific receptors, may be used (see Figure 5). Such sensor arrays may be prepared for sensing multiple target substances using photo-patterning known to those skilled in the relevant art. An additional advantage of an embodiment such as Figure 5 is that degradation of device sensitivity due to long lead and contact resistance can be reduced by integrating associated circuitry (100, 102, 104) on a common substrate with on-chip interconnects. Such on-chip interconnects further minimize test solution contamination associated with lead contacts and electrodes.

While generally the particle or, target substance, 64 will be a chemical or biochemical, the present invention can also test for the presence of photons, the effects of force, magnetic fields, electric fields, and the like. As used herein, "chemicals" includes not only solutions, molecules, ions, and atoms, but also subatomic particles, such as electrons. As used herein, "biochemicals" includes not only biochemical compounds such as sugars, fats, proteins, etc. but also polymers such as proteins, nucleic acids, glycosaminoglycans, and the like, and encompasses microorganisms and fragments thereof, such as bacteria, viruses, and protozoa.

With reference to Figure 2B, preferably, applicant's depletion mode EIFET structure includes a lightly doped n-epi layer 57 (e.g., the built-in channel) fabricated on a low resistivity p substrate 51, wherein N+ regions 53, 55 form the device source and drain regions. The gate 60 structure is somewhat similar to a JFET with a MOS gate, but without metalization. Like a JFET, the device has a built-in channel 56 that, operating in depletion mode, is normally on, with the depth of the built-in channel (and thus channel conductance) controllable via a reverse bias coupled between source 53 and substrate 51.

While the present device 50 provides good sensitivity, sensitivity enhancement strcutres can provide yet additional sensitivity. Typically the topgate bias causes depletion or accumulation of the channel region 56 immediately under the topgate 60. (Alternatively, if the device 50 incorporates an inversion channel, topgate bias influences the extent of the inversion, e.g., channel conductance.) The backgate is preferably reverse biased to permit the device to operate in a highly sensitive regime under pre-attachment conditions. A relatively small change in a gate attached charge modulates channel depletion width 56 and channel conductance. This condition also requires the largest restoration backgate potential 74, 234 (Figures 3A, 6A) to reset the device 50 to the pre-attachment co· ·ition.

With reference to Figures 3A and 6A, preferably a device according to the present invention uses backgate bias 74, 234 to restore device operating characteristics to a preattachment condition, where a device operating parameter is selected as a reference value. For example, the reference parameter can be source-drain current, pinch-off voltage, transconductance characterized by AC signals, source-drain voltage for constant source-drain current, etc.

In this operation mode, the device backgate 62, 270 is biased to restore the preattachment reference characteristic. Thus if attachment increases channel depletion 62, 271 in the depletion mode, a preattachment reverse backgate bias would require reduction to restore preattachment operating drain current, where source-drain voltage was held constant. The shift in backgate bias to restore the preattachment condition provides a measure of attachment. In depletion mode operation, the backgate voltage preferably almost pinches off the channel 56 before attachment of charge to the topgate. This bias regime permits the largest percentage change of channel resistance and current to be determined, and advantageously requires the largest restoring back bias voltage.

The use of a measurable backgate bias to a restore preattachment condition permits automation using circuits and components known to those skilled in the relevant art. For example, a voltage divider providing a controllable output voltage coupled to the backgate could be used with a simple feedback circuit 240 to reestablish drain-source current, the amount of restoration voltage providing a measure of the attachment condition.

A reference pulsed drain-source current 240 could also be maintained with a pulsed backgate voltage 234. The resultant pulsed current could be detected and used to adjust, via feedback, the pulsed backgate voltage to restore the current to the pre-attachment value. Alternatively, a pulsed AC drain-source current dependent upon device transconductance could be used. The use of AC signals such as time dependent backgate pulse voltages advantageously permits DC blocking capacitors 27 to be used, AC amplification 212 to be incorporated free from DC drift influences, and allowing AC filters to reject noise to enhance device sensitivity.

Sensitivity enhancement is attained because for PN junctions (e.g., a backgate pn junction 270, or a reverse biased channel-substrate 270), the amount of depletion resulting from a small reverse bias voltage change is generally dependent upon the initial reverse voltage magnitude. For example, for a one-sided abrupt junction whose n-side is doped at $10^{15}/cm^3$, the depletion width is approximately 5 μm at 20 V reverse bias, and is about 6 μm at 40 V reverse bias. It is this nonlinearity that may be used to increase measurement sensitivity in a device employing a suitable backgate.

Thus, substantially reverse biasing the backgate requires a relatively large incremental change in reverse bias (compared to an initial lower reverse bias potential) to provide a given change in depletion width necessary to reset or reestablish preattachment channel conductance. Where source-drain output current is to be maintained, the backgate potential is modulated to restore the conducting channel to a preattachment conductance. Whether an increase or decrease in backgate reverse bias 74, 234 is required will depend on whether attachment increases or decreases depletion or accumulation beneath the gate region 60. The magnitude of voltage change 74, 234 to restore preattachment channel condition increases with increasing initial backgate bias, thus enhancing device detection sensitivity to attachment.

If this incremental backgate bias change 74, 234 is then coupled to the gate of another FET device (FET 2), a much larger output signal 212 is available from FET 2 than would have initially been present in the first device 50 due to attachment induced conductance modulation effects. In this fashion, device attachment sensitivity is increased. If the backgate 62, 270 is operated in AC pulsed mode, AC amplification of the signal delivered to the second device can yet further enhance sensitivity.

Device 50 can be designed to enhance sensitivity by selection of dopant and doping profiles for the pn junction comprising the backgate region 62, 270. Where both regions are homogeneously doped, depletion extent will be the same on either side of the junction. If however one region 273 is more heavily doped than side 274, then 273's side side will experience a shallower depletion extent, depletion being inversely proportional to the homogeneous doping ratio. For example, if the junction's n side is 10^{+4} more heavily doped

than the p side, applying a given incremental reverse backgate bias results in about 10^{-4} of the incremental bias falling across, and modulating, the n region. Stated differently, a substantially larger incremental voltage must be applied to the backgate 62, 270 to restore a preattachment state than would be the case if the two sides of the junction were equally doped. In essence, the two sides of the pn junction perform a voltage division function, providing a small fraction of the total backgate bias change to the channel side of the backgate junction. Non-linear junction doping can also enhance this desirable sensitivity enhancement feature of a backgate, according to the present invention.

Other back gate junctions may be used with the present invention to enhance target detection sensitivity. A P-I-N backgate substantially enhances sensitivity using a large intrinsic region, and dropping a large fraction of applied backgate restoring potential across the I region. Thus, a very small fraction of the backgate bias modulates the n-channel width, leading to especially large sensitivity enhancement.

Figure 3A depicts a first preferred "wet" measurement embodiment wherein a device 50 contacts a test solution 52 within a container 54, such as a microtiter plate. In operation, film 90 and receptor 66 portion of device 50 are exposed to the test solution 52, allowing any target substance 64 present to bind or attach. Because substance 64 has an associated electrical charge, post-attachment electrical charges will be present at receptors 66 that can affect the performance of device 50.

As noted, semiconductor device 50 will generally include at least one field effect region wherein the conductivity of a channel 56 is modulated is response to electrical or charge activity on one or more gates 60, 62. Such charge (from bound target 64, for example) creates an electric field that operates through the thickness Δt of a typically dielectric insulating layer 58 (e.g., SiO_2) and terminates on charges within the semiconductor, thereby altering the semiconductor channel region 56. The magnitude and quantity of such bound charges alters the resistance of the conducting channel.

Alternatively, the device may not employ a conducting channel. A sensor field effect device could be implemented as a capacitor device that senses chemical associated charge attachment by modulating capacitance change, which change could be sensed by including the capacitor in an appropriate electronic circuit, an RC oscillator, for example.

For a field effect transistor ("FET") device, the resultant electric field modulation of the channel conductance (or resistance) alters the typically drain-source current flow in the channel. Charge binding thus alters one or more otherwise quiescent characteristics of device 50, which alteration may be sensed and measured, qualitatively and/or quantitatively, using equipment

70. The measurable characteristics may include, without limitation, drain current, change in threshold voltage, pinchoff voltage, gate-source voltage, transconductance, conductance, gate-source capacitance, gate to substrate capacitance, back gate capacitance, transconductance threshold voltage "DCBD", and gated bipolar devices), bipolar current gain (DCBD), transconductance (DCBD), and change in source voltage for constant drain current.

For example, the attachment of a target-originating charge sheet results in an associated electric field generated across a thickness of insulator material, which results in a threshold voltage shift in an insulator gate field effect device. The resultant incremental threshold voltage change alters the device's operating characteristics, which characteristics may be used to influence an associated circuit to provide an enhanced measurement signal representing attached charge. Such measurement may represent, for example, channel resistance, switching time, oscillator frequency (where the device is incorporated in an oscillator circuit), channel transconductance, output current into a bipolar transistor base, etc. Further details regarding field effect theory and modelling may be found in a number of standard treatises, including "Device Electronics for Integrated Circuits", 2nd ed., by Muller and Kamins, published by John Wiley.

In the configuration of Figure 3A, attachment measurements are made while device 50 is still in the solution 52, typically at the time of attachment, or in some other solution (here shown as 52 also) whose pH, temperature, chemical composition, etc. may differ from test solution 52. As shown, during measurement device 50 is coupled to measurement equipment 70, which preferably includes one or more measuring instruments 72 such as an oscilloscope, current probe, semiconductor parameter analyzer or curve tracer, capacitance measuring instrument, specially designed instruments sensitive to the sensor parameters of interest, and the like. Equipment 70 also includes a variable power source 74 that is preferably coupled to device 50's bottom gate 62. Optionally, equipment 70 can include an additional power source 76, coupled to device 50, for establishing a pre-binding quiescent state.

When a target substance 64 binds to a receptor 66, the associated substance charge and/or contact potential will alter device 50's conductance state. For example, depending upon the mode of device 50, a binding condition can be associated with an increase or decrease in channel conductance, a change in drain current or pinch-off voltage or threshold voltage, a change in transconductance, in channel width or depletion, and so on. One or more such characteristic changes may be measured by equipment 70 to provide a signal corresponding to attachment.

Preferably the variable power source 74 is adjusted, post-attachment, to restore whatever pre-attachment quiescent condition existed for device 50. Equipment 70 can measure the amount

of restoration bias required from power source 74 to provide an increased measure of the attachment. The compensating or restoration bias can be amplified, e.g., by amplifier 78, and the amplified signal coupled to one or more additional components 80. Component 80, in turn, could include a second device 200, coupled to perform a cascode amplification function upon the amplified restoration bias signal. If desired, the compensation bias signal from potential source 74 may be coupled to a feedback circuit 82 to automatically provide and maintain proper restoration bias (see also Figures 6A and 6B). The design of such feedback circuitry is well known to those skilled in the relevant art, and thus detailed information is not here presented.

With further reference to Figure 3A, as indicated by capacitor 82, the restorative bias from potential source 74 could in fact be an AC coupled pulse train, wherein one level corresponds to "pre-binding" and a second level of the pulse train corresponds to "post-binding". Because such pulse train signal is AC, capacitor 82 can decouple undesired effects of power source. This facilitates AC amplification of the detected signal, reducing susceptibility to problems associated with drift in DC amplifiers, power sources, etc. Further, capacitor 82, coupled in series with the bottom gate 62 as shown, determines the extent an applied AC compensating reverse bias from power source 74 affects device 50's channel conductance, or other operating characteristics. Voltage division, e.g., with a capacitor voltage divider, can also provide a "sensed" voltage gain, wherein a small fraction of the power source is used as a restorative potential, with the total power supply potential being measured to indicate the amount of gate charge attachment to be sensed.

The embodiment of Figure 3B is similar to what has been described except that while binding occurs in solution (as in Figure 3A), dry measurements are made. In practice, device 50 is exposed to the test solution 52 potentially containing the target substance 64. It is understood that in this embodiment, equipment 70 need not be present when or where device 50 is exposed to the target substance.

Materials needed for measurement enhancement and any confirmational testing need not exist at the time and place that testing occurred (only at the measurement site). This is advantageous, especially if the testing-binding site is in a remote location, or a location not amenable to storing enhancement materials under controlled conditions.

Applicant has discovered that such dry measurement advantageously eliminates the ion shielding problem so prevalent in the prior art. According to the present invention, removing the device 50 from an ionic solution 8 before testing facilitates elimination of the external reference electrode and attendant gate bias problems, drift and potential chemical contamination so prevalent in the prior art. In the present invention, attached surface charge is sensed directly.

Once a sensor 50 with bound target matter 64 is removed from an ionic test solution 52, the charges associated with the bound matter are no longer shielded by ions in the no longer present solution. As a result, a net charge is manifested at layer 60 generating an associated electric field across the underlying insulator layer 58. This charge and the attendant electric field attract oppositely charged free carrier charges from the semiconductor substrate 56 (or repel like polarity free carrier charges), resulting in opposite charges at or near the surface of the substrate 56. It is to be noted that the effects of contact potential at the sensor gate are generally different than the effects of bound charges.

Contact potential and charge attachment influences on a sensor, arising from target material attachment, may be separated by comparing the influence of target material attachment on the topgate region 66 of two sensor devices 50, 50′ identical except for their insulator thickness 91. Separating the effects of these two phenomena occurs because while the attached charge generates an electric field across an insulator independent of insulator thickness, the contact potential induced electric field is inversely dependent upon insulator thickness. Thus, making two independent measurements of two unknowns permits identifying charge attachment effects, and contact potential effects upon sensor device 50.

Where the desired information is a direct measurement of target material concentration, binding rate of the target material to a receptor, dissociation rate, binding energy, etc., the test may be interrupted before steady state is achieved.

According to a preferred embodiment depicted in Figure 5, substrate 56 can include an on-chip pH sensor 100 and storage mechanism 102 for memorializing the test solution's pH value. The on-chip pH sensor 100 itself may be implemented as a device, according to the present invention, and may include the various enhancement techniques described herein.

Further, various confirmational devices, devices for measuring cofactors, and devices for measuring other chemicals of interest and for measuring chip receptor condition and integrity can be included on the same semiconductor chip. Preferably the sensor system further includes an on-chip mechanism 104 for measuring and storing the pre-attachment quiescent conditions of one or more devices (e.g., 50A, 50B, etc.) fabricated on the same substrate. For ease of illustration, Figure 5 does not depict the couplings between device 104 and the various sensors.

As depicted by Figure 5, the sensor system 106 can include measurement circuitry, e.g., 104, for each sensor device, and circuitry providing error alerts where, for example, receptor integrity, storage conditions, etc., are in question. Such on-chip circuitry can include a temperature sensor to monitor incubation conditions present during the chemical reaction of

interest (including attachment and dissociation), as well as a clock measuring reaction or dissociation process times. Thus, system 106 can measure physical parameters including reaction temperature, reaction time, solution pH, and ionic concentration.

In the generalized embodiment of Figure 5, note that it is not necessary that each sensor be identical, either in size, or in the density and/or number of receptor types 66, 66'. For example, sensor 50F includes no receptors and may be coated with an inert material, and may be used as a calibration sensor in that the "before" and "after" binding characteristics of sensor 50F should be the same. Confirmational testing excluding a non-specific reaction with device 50F's inert material could be achieved by providing another sensor whose outer film should also be non-reactive with the test solution contents. "Before" binding data from sensor 50F may be stored in device 104 for comparison to the sensor's "after" binding data. If the two sets of data do not agree, a user would know to suspect the integrity of data from sensors 50A-50E. For example, the array 106 of sensors may have been damaged at some time.

The embodiment of Figure 5 also promotes confirmatory testing not merely of the devices themselves, but of various target materials. For example, device 50B has a greater density of the same type of receptors than does device 50A. Therefore confirmation of binding the appropriate target material for receptors 66 would be indicated by a proportionally greater binding effect upon device 50B contrasted with device 50A.

Further, by providing some devices with only a first type of receptor (e.g., device 50A), and some devices with only a second type of receptor (e.g., device 50C), a differential analysis of a binding event may be made. For example, a target substance suitable for binding only with receptors 66 should produce a measurable change in device 50A, but not in device 50C. Further, a device such as device 50E may include both sensor types to provide still further confirmatory information as to the nature of what the binding substance is. Additional confirmational data may be acquired by re-exposure of a previously exposed test device to a different temperature, different pH, different chemicals, etc., providing known results for the target material. For example, disassociation rate of nucleic acid components with temperature, dissociation with pH change, charge change with pH change, charge sign change with pH change, disassociation as a function of different receptor binding sites, etc., provide useful information, especially as to whether what bound to the receptor of interest was in fact the desired target material.

Differential analysis could also be performed by exposing two identical sensors to a target material for two different time lengths, each shorter than the time known to be required for the target-receptor reaction to complete. Charge measurement for each device provides data for

target material reaction (or attachment) rate, and concentration in the test solution, data useful to provide species identification.

Whether measurements are conducted wet (as depicted in Figure 3A) or dry/quasi-dry (as depicted in Figure 3B), the bound charges result in an electric field that can modulate the conductance of the device semiconductor channel (if not neutralized by shielding, etc.). This modulation permits detection using suitable equipment 70, according to the present invention. While the description thus far has been with respect to a sensor 50 such as depicted in Figures 3A and 3B, according to the present invention, other semiconductor field effect sensors including the embodiment of Figure 4 (and devices functionally similar thereto) could also be used.

The present invention also facilitates obtaining sequence information of polymeric molecules such as DNA, RNA, glycoconjugates, polypeptides, and so forth. Another preferred sensor embodiment that is very well suited for detecting binding attachment in a sensor application is depicted in Figure 4. Device 50' is a so-called distributed channel bipolar device ("DCBD"), similar to what is disclosed in U.S. Patent No. 4,885,623 to James Holm-Kennedy, et. al. (1989). The DCBD structure of device 50' includes FET components gate oxide insulation layer 58', an appropriate moisture blocking layer 90', gate 60', drain 100, channel region 56', as well as bipolar components base 104 and emitter 106. While Figure 4 depicts a DCBD device with vertical architecture, alternatively, a DCBD device could be fabricated with lateral architecture, or a combination of vertical and lateral architecture. Similar to what was set forth above with reference to device 60 of Figures 3A and 3B, in sensor applications, the gate 60' supports an moisture blocking protective film 90' to whose outer surface primary receptors 66' are attached.

As described in detail in said U.S. Patent No. 4,885,623, depending upon mode of operation, the DCBD may be treated as an FET device with a bipolar source or drain that may be distributed. The DCBD may also be treated as a bipolar device with an FET emitter or collector that may be distributed, or as a gated bipolar device. The DCBD may be operated in enhanced or depletion mode. Further, vertical and lateral bipolar devices can be incorporated in a DCBD device. The FET portion of a DCBD device may incorporate a MESFET, JFET, or IGFET structure.

DCBD devices are especially well suited for sensor wet and dry testing, according to the present invention. DCBD devices exhibit high transconductance and current gain sensitivity to relatively small changes in gate voltage or charge, resulting from the binding of a target substance to the device. DCBD devices are also very sensitive to small changes in base current, under certain operating conditions. Further, techniques may be employed to enhance

various DCBD parameters in response to target binding. Such enhanceable parameters include bipolar gain, gate and base current dependent current gain, heterogeneous channel behavior, effective gate area and gate shape, and transconductance threshold voltage effect. Transconductance measurements with device 50' are especially advantageous because a null may be detected using alternating current ("AC") amplifiers, operating at high gain. Electrophoresis applications are also applicable to sensor devices such as the present invention.

With reference to Figures 6A and 6B, two embodiments of feedback for sensor reset and sensitivity enhancement measurement approaches are shown. In Figure 6A, sensor 50 is biased by a pulsed backgate bias supply 234, to reset or restore device 50 to its preattachment condition, while measurement system 230 monitors a device operating parameter sensitive to charged target attachment. For ease of illustration, parameter measurement system 230 is indicated generally, without a specific coupling 232 to the device to illustrate that the chosen parameter(s) may be varied, and can include current, voltage, transconductance, etc. The sensed parameter provides a feedback signal to feedback system 240 that, in response, adjusts the backgate operating voltage to restore the preattachment conditions.

In Figure 6A, the reset voltage from supply 234 is coupled to the input of a second device, FET 2, selected for high gain (e.g., a thin gate oxide device). In this cascode circuit, output current I_d 212 from sensor device 50 is amplified by FET 2, whose FET 2 output may be used to detect the target attachment event. Further, this output signal 212 may be processed with circuit 214 to provide additional information, and/or may be coupled to a feedback circuit 240 (shown in phantom) for restoring preattachment device 50 conditions. If desired, FET 2 may be a component in a circuit whose performance is affected by FET 2's change in channel resistance responding to attached charge at device 50. For example, if FET 2 affects the frequency of an oscillator 210, attachment at device 50 may be sensed by monitoring frequency change at oscillator 210.

In Figure 6B, a constant current is maintained in device 50, whereupon a certain voltage is seen across device backgate 270. Charge attaching to the topgate 272 of device 50 will induce a backgate voltage change, which change provides a measure of the attached charge. The current I_d may be AC pulsed, which allows AC amplification 237 without problems associated with DC amplifier drift.

In this example, the net charge upon device 50 from target 64 has been increased, including charge effects from the additional attachment of receptors to the "sandwich". Numerous other means of enhancing or amplifying the signal generated by target binding are available to those skilled in the relevant art. For example, as depicted in the right-hand portion of Figure 7B, a conglomerate may be used in the third sequence, wherein device 50 could be exposed to material having substantially more charge than target 64. For example, such material might

include a DNA chain (or fragment) 112 attached to a target substance 64X that will bind to the receptor 66X. In this fashion, a substantially larger net charge attaches to the field effect sensor device, resulting in a larger signal. It is understood that charge associated with receptor 66 and target 64 may be additive or subtractive, as may also be the case with material 66X, 64X and 112.

Figure 7C depicts the use of beads as an alternative (or additional) method of passively enhancing device sensitivity. Beads 114 may have a variety of shapes, are commercially available in sizes ranging from about 0.1μ to several μ, and are glass or polymer, e.g., materials affording many binding sites for a plurality of secondary receptors 66'. Receptors 66' have similar selective reactivity characteristics as the primary receptors 66, and may in fact be identical thereto. Beads, like conjugates, are commercially available and are frequently made from gold, biotin, ferritin, and the like, and may be made from other chemicals such as nucleic acid chains and antibody chains. In a second sequence, after target 64 has bound to primary receptor 66, the device 50 is exposed to a typical solution containing conjugated receptors 116.

With reference to Figures 8A-8C, the drain current (Ids) and drain-source voltage (Vds) for sensor 50 was measured after the application of various biolayers. For the data shown in these figures, incubation was 27°C with a 10mM MOPS buffer employed to maintain pH ≈ 7. With reference to Figure 8B, for example, the device was tested before and after application of a parylene film 90 (bar graphs a and b). After stabilizing the device in a MOPS buffer solution measurements were again taken (bar graph c). A receptor layer (receptor:IgG) was applied, and measurements taken (bar graph d), after which the device was exposed to a BSA blocking agent and measured (bar graph e). The utility of the BSA blocking layer is shown by bar graph f (Figure 8B), wherein the device was exposed to a non-specific antibody. As shown by bar graphs e and f, there was relatively little change in charge from non-specific bindings, which indicates BSA suppression of non-specific binding. Next the device was exposed to a desired target substance (antigen: Goat anti-IgG), and a change in charge (ΔQ) was noted (bar graph g). Finally, the device was reexposed to receptor material (IgG), which resulted in essentially the same charge change (ΔQ). It is noted from Figures 8B and 8C that different chemical exposure and reactions can result in charge attachment of different polarities, as indicated by the direction of the bar shift after exposure to subsequent chemical exposure.

The data for bar graph a in Figure 8C depicts a denatured device 50. After denaturing, the device was exposed to a buffered MOPS solution (bar graph b), which altered the effective charge on the device. Thereafter in a suitable incubation environment, receptors were attached (bar graph c), which in this case altered the gate attached charge. Exposure to a BSA

environment (bar graph d) slightly changed the charge, and exposure to the target material (bar graph f) resulted in a desired perceptible charge change ΔQ.

Automated testing is readily implemented using the invention, which can include on-chip testing circuitry (see Figure 5). Dry testing particularly promotes automated procedures, where the bound sensors may be automatically delivered to a test fixture (e.g., a test probe), where test information is read and the test results stored for automatic computer processing.

While the present invention has been described with reference to a few specific embodiments, the description is illustrative of the invention and is not to be construed as limiting the invention. Various modifications may occur to those skilled in the art without departing from the true spirit and scope of the invention as defined by the appended claims.

WHAT IS CLAIMED IS:

1. A semiconductor device (50) for sensing without a reference electrode the presence of a desired particle (64) in a first solution (52), the device comprising:

a substrate (51);

a topgate region (60), including a chemically selective material (66), producing a desired first electrical signal upon attachment of said desired particle (64), said topgate region (60) overlying said substrate (51);

a field effect channel region (56) in said substrate at least partially underlying said topgate region;

a backgate region (62) adjoining said field effect channel region (56);

wherein said field effect channel region (56) is in field communication with said topgate region (60) and with said backgate region (62), said channel (56) being modulatable in conductance by an electrical signal from said topgate region (60) and/or from said backgate region (62);

wherein said desired first electrical signal modulates said channel's (56) preattachment conductance, providing an output signal from said device (50) indicating attachment.

2. The device of claim 1, wherein said field effect channel region (56) has channel characteristics similar to at least a chosen one of the group consisting of a junction field FET, a non-metalized MOSFET, a non-metalized MISFET, an ISFET, an inversion FET, a DCBD, a heterojunction FET, a transistor, and a capacitor.

3. The device of claim 1, further including means, coupled to said backgate, for enhancing an output signal indicating attachment.

4. The device of claim 3, wherein said means includes first means for monitoring a parameter of said device, and bias means for adjusting a bias at said backgate after attachment to restore said parameter to a preattachment state, wherein the bias adjustment provides a measure of attachment.

5. The device of claim 1, wherein said backgate comprises a semiconductor junction whose dopant concentrations and/or doping profiles are selected to cause a desired small fraction of an applied backgate bias voltage to modulate said channel conductance.

6. A system for sensing the presence of a desired particle (64) in a first environment (52), the system comprising:

a substrate (51);

at least one semiconductor sensor (50), on said substrate (51);

first electrical means (100), on said substrate (51), for measuring at least one parameter of said first environment (52);

second electrical means (102), on said substrate (51), coupled to an output of said first electrical means (100) for memorializing at least one parameter of said first environment (52).

7. A method for detecting and measuring the presence of a desired particle in a first solution by its attachment to a field effect device, the method comprising:

exposing the field effect device to said first solution, wherein said field effect device includes a surface adapted to bindingly attach to said desired particle, said attachment altering at least one parameter of said device from a preattachment state;

at least quasi-drying said device; and

measuring, in a second environment, said at least one parameter to determine whether attachment of said desired particle occurred.

8. The method of claim 7, wherein said second environment is selected from the group consisting of air, and a second solution substantially different in pH, temperature and/or chemical composition from said first solution.

9. The method of claim 7, further including the step of providing means for modify charge associated with said attached target.

10. The method of claim 7, wherein a chosen region of said surface of said device is receptive to said attachment, said chosen region resulting from a chosen step selected from the group consisting of (a) embedding a shielding layer (214) in said device (50) during device fabrication, which layer shields said device save for a region underlying said chosen region, and (b) forming a pattern mask of inert material (210) on all of said surface save for said chosen region thereof.

AMENDED CLAIMS

[received by the International Bureau on 12 March 1993 (12.03.93); original claims 1-5 amended; other claims unchanged (2 pages)]

1. A semiconductor device (50) for sensing without a reference electrode the presence of a desired particle (64) in a first solution (52), the device comprising:

a single substrate (51);

a topgate region (60), including a chemically selective material (66), producing a desired first electrical signal upon attachment of said desired particle (64), said topgate region (60) overlying said substrate (51);

a field effect region (56) in said substrate at least partially underlying said topgate region;

a backgate region (62) adjoining said field effect region (56); and

means (50', or 62 or 74 or 82 or 200 or 212 or 230 or 234 or 240 or 270) for enhancing measurement sensitivity of said semiconductor device (50) to attachment of said desired particle (64);

wherein said field effect region (56) is in field communication with said topgate region (60) and with said backgate region (62), said field effect region (56) being modulatable in conductance by an electrical signal from said topgate region (60) and/or from said backgate region (62);

wherein said desired first electrical signal modulates said field effect region's (56) preattachment conductance, providing an output signal from said device (50) indicating attachment.

2. The device of claim 1, wherein said field effect region (56) is a field effect channel region having channel characteristics similar to at least a chosen one of the group consisting of a junction field FET, a non-metalized MOSFET, a non-metalized MISFET, an ISFET, an inversion FET, a DCBD, a heterojunction FET, a transistor, and a capacitor.

3. The device of claim 1, wherein said means for enhancing measurement sensitivity includes electrical means, coupled to said backgate, for enhancing an output signal indicating attachment.

4. The device of claim 3, wherein said electrical means includes first means for monitoring a parameter of said device, and bias means for adjusting a bias at said backgate after attachment to restore said parameter to a preattachment state, wherein the bias adjustment provides a measure of attachment.

5. The device of claim 1, wherein said backgate comprises a semiconductor junction whose dopant concentrations and/or doping profiles are selected to cause a desired small fraction of an applied backgate bias voltage to modulate said field effect region conductance.

6. A system for sensing the presence of a desired particle (64) in a first environment (52), the system comprising:

a substrate (51);

at least one semiconductor sensor (50), on said substrate (51);

first electrical means (100), on said substrate (51), for measuring at least one parameter of said first environment (52);

second electrical means (102), on said substrate (51), coupled to an output of said first electrical means (100) for memorializing at least one parameter of said first environment (52).

7. A method for detecting and measuring the presence of a desired particle in a first solution by its attachment to a field effect device, the method comprising:

exposing the field effect device to said first solution, wherein said field effect device includes a surface adapted to bindingly attach to said desired particle, said attachment altering at least one parameter of said device from a preattachment state;

at least quasi-drying said device; and

measuring, in a second environment, said at least one parameter to determine whether attachment of said desired particle occurred.

8. The method of claim 7, wherein said second environment is selected from the group consisting of air, and a second solution substantially different in pH, temperature and/or chemical composition from said first solution.

9. The method of claim 7, further including the step of providing means for modify charge associated with said attached target.

10. The method of claim 7, wherein a chosen region of said surface of said device is receptive to said attachment, said chosen region resulting from a chosen step selected from the group consisting of (a) embedding a shielding layer (214) in said device (50) during device fabrication, which layer shields said device save for a region underlying said chosen region, and (b) forming a pattern mask of inert material (210) on all of said surface save for said chosen region thereof.

STATEMENT UNDER ARTICLE 19

Sakai, et al., U.S. Patent no. 4,961,833 was cited against originally filed claims 1-6, while Guckel, U.S. Patent no. 4,180,771 was cited against originally filed claims 1-12.

Applicant has amended claims 1-5. Provided herewith are substitute pages 22 and 23 containing claims 1-12, wherein claims 1-5 have been amended as above.

Amended claim 1 recites that the claimed device comprises a single substrate. This limitation is supported by any of Figures 2A, 2B, 3A, 3B, 4, 5, 6A, 6B. By contrast, Sakai, et al., U.S. Patent no. 4,961,833 discloses a device having first and second substrates (12, 14), separated by an oxide layer (13). See Sakai, et al., Figure 1. Amended claim 1 refers to region (56) as a field effect region, which it is, rather than as a channel, which it may be. See for example, the Specification, page 13, line 27.

Claim 1 has also been amended to require that the device include means for enhancing measurement sensitivity. This limitation is supported, inter alia, by Figures 2A, 2B, 3A, 3B, 4, 5, 6A and 6B. See also the accompanying text in the Specification at pages 11-13, 15, 16, and 19.

Claims 2-5 have been amended to conform to the nomenclature in amended claim 1.

The Sakai, et al. Reference Distinguished:

Sakai, et al. was cited against originally filed claims 1-6. Sakai, et al. discloses a structure wherein every embodiment has two substrates separated by an oxide layer. As noted, the presently claimed invention set forth in claims 1-5 requires but a single substrate. Further, the means for enhancing sensitivity now set forth in amended claim 1 is neither disclosed nor suggested in Sakai, et al.

Claim 6 requires, inter alia, first and second electrical means (100, 102), which means are neither disclosed nor suggested in Sakai, et al.

Applicant therefore submits that the invention set forth in claims 1-6 is neither anticipated by nor rendered obvious by Sakai, et al.

The Guckel Reference Distinguished:

Guckel was cited against originally filed claims 1-12. Amended claim 1 requires, inter alia, means for enhancing measurement sensitivity. Applicant submits that such means are neither disclosed nor suggested by Guckel. Further, the preamble to claim 1 recites that the claimed device is used for sensing without a reference electrode. Guckel's device includes such a reference electrode (see Guckel Figure 1, element 24), although Guckel appears to suggest that the reference electrode need not be used at all times.

Applicant submits that amended claim 1 is neither disclosed nor suggested by Guckel. Similarly, dependent claims 2-5 recite elements not disclosed or suggested by Guckel.

Claim 6 recites a system that includes, inter alia, first and second electrical means (100, 102), which means are neither disclosed nor suggested by Guckel, or a combination of Guckel and known prior art.

Method claims 7-10 include, inter alia, the step of at least quasi-drying a field effect device after attachment has occurred and before measurements are made. Further, claims 7-10 require that measurement occur in a second environment. Claim 8, for example, states that the second environment may be air. Claim 9 requires the further method step of providing means for modifying the charge associated with an attaching target.

Applicant submits that Guckel alone or in combination with known prior art does not disclose or suggest the method set forth in claims 7-10.

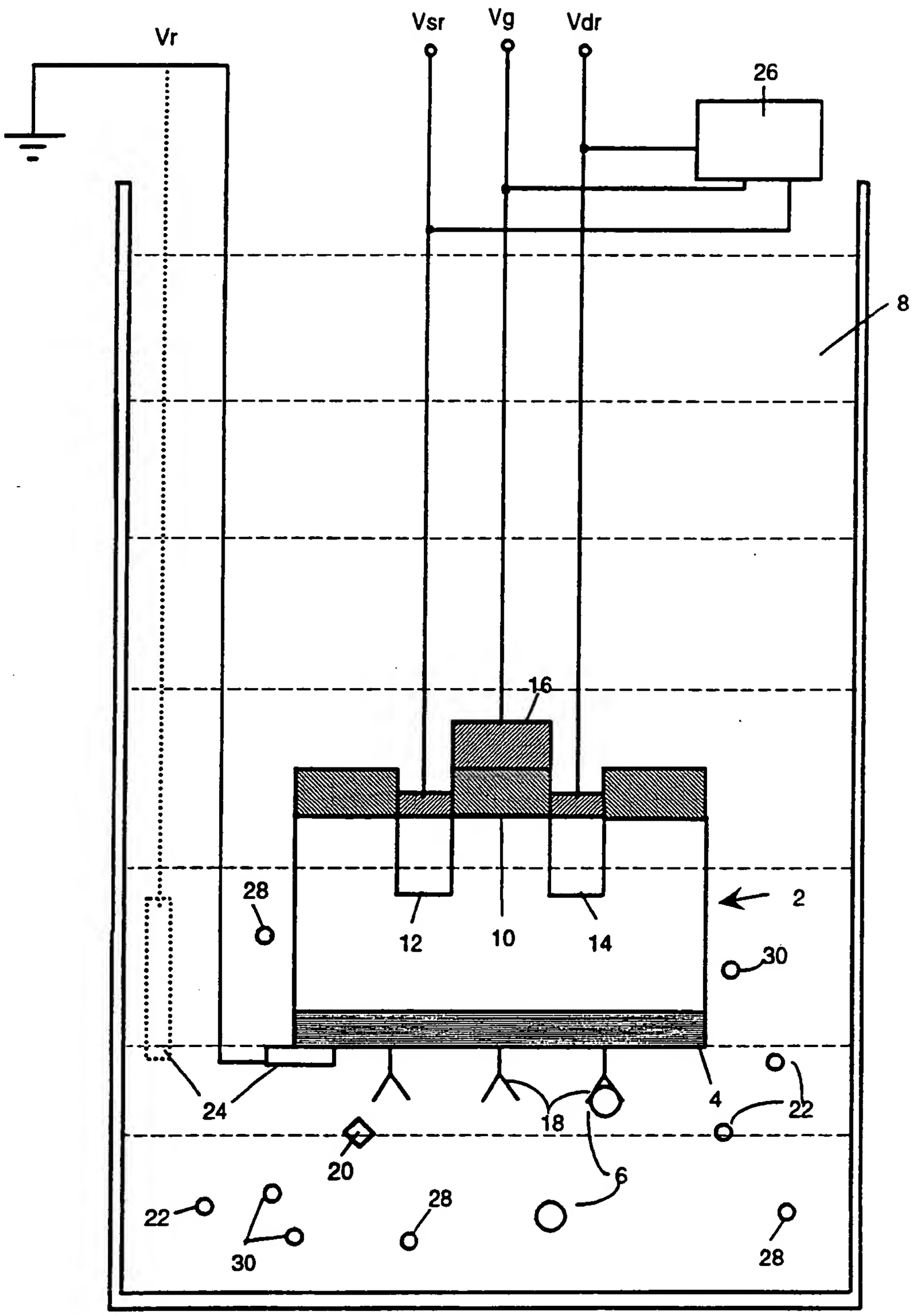


FIGURE 1

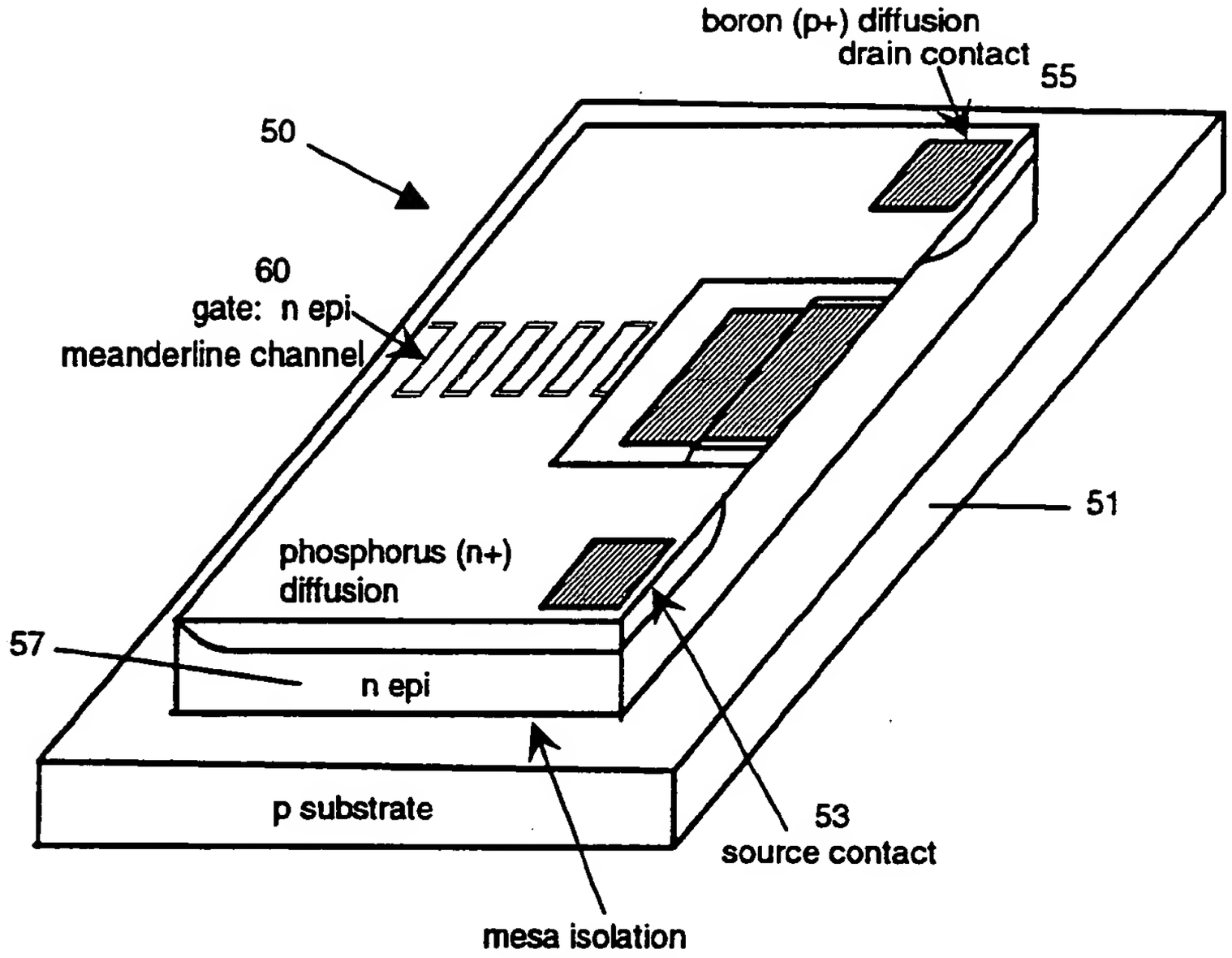


FIGURE 2A

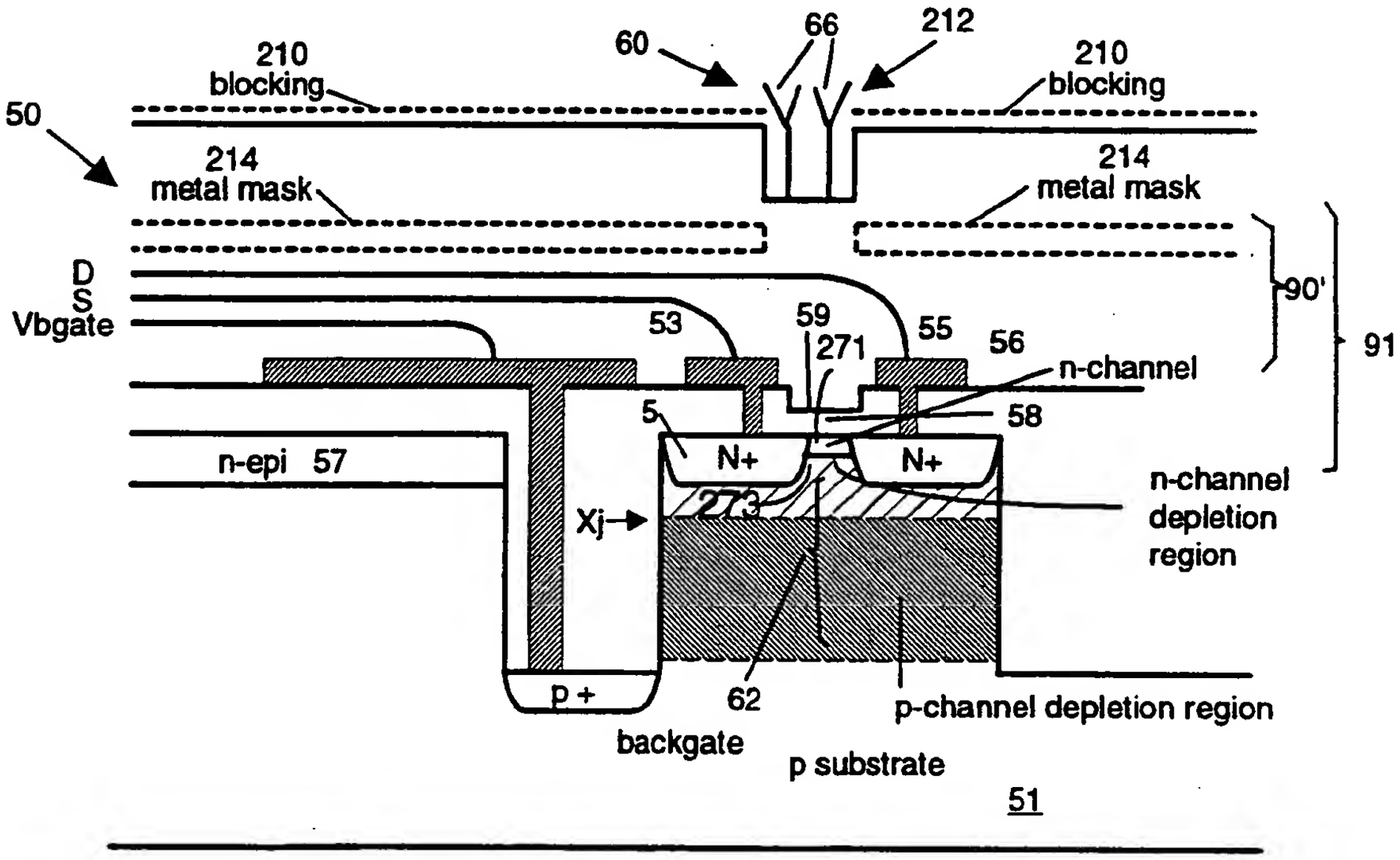
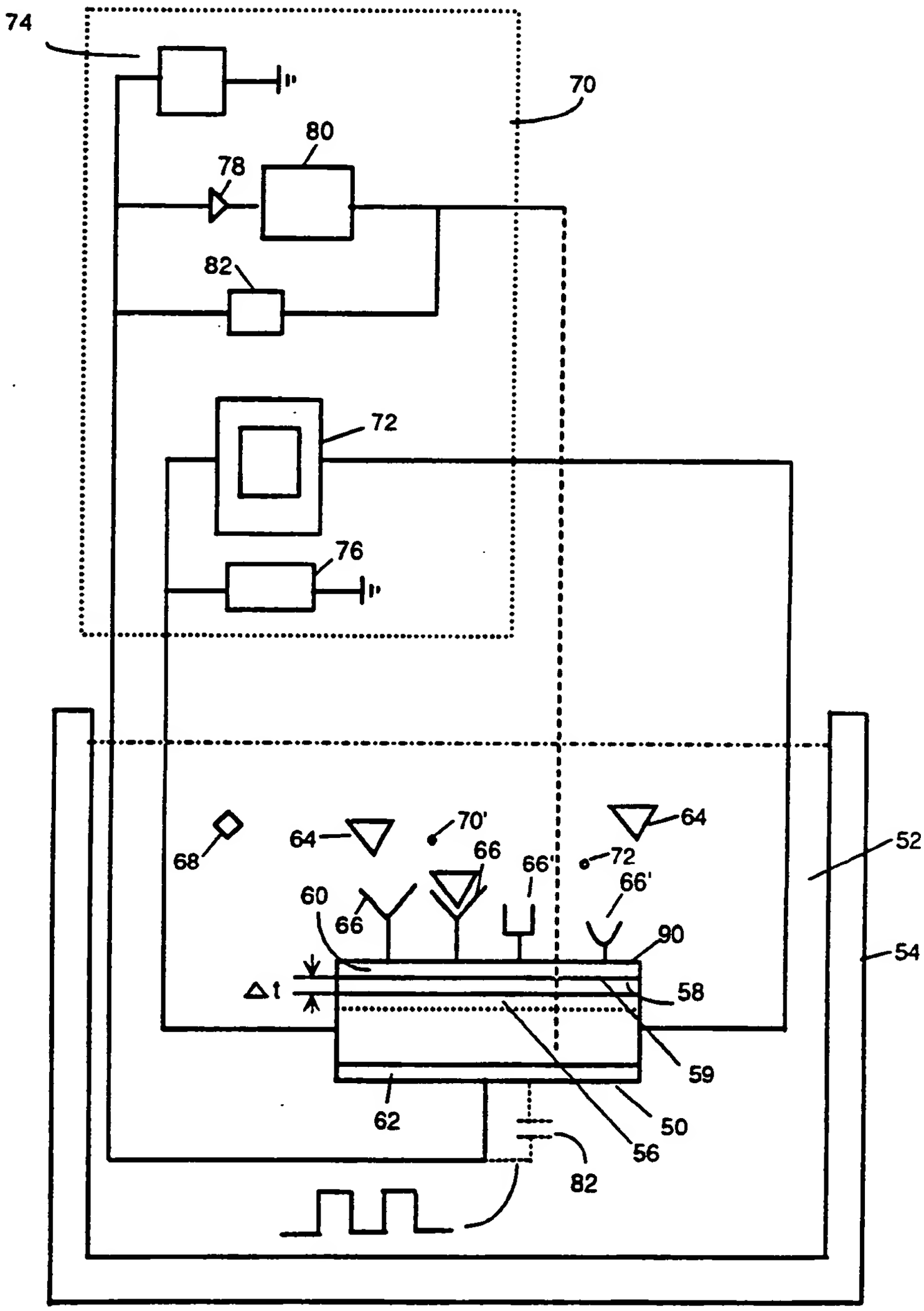


FIGURE 2B


74
70
80
78
82
72
76
68
64
70'
66
66'
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72
66'
60
66
90
52
54
Δt
58
59
62
50
56
82

FIGURE 3A

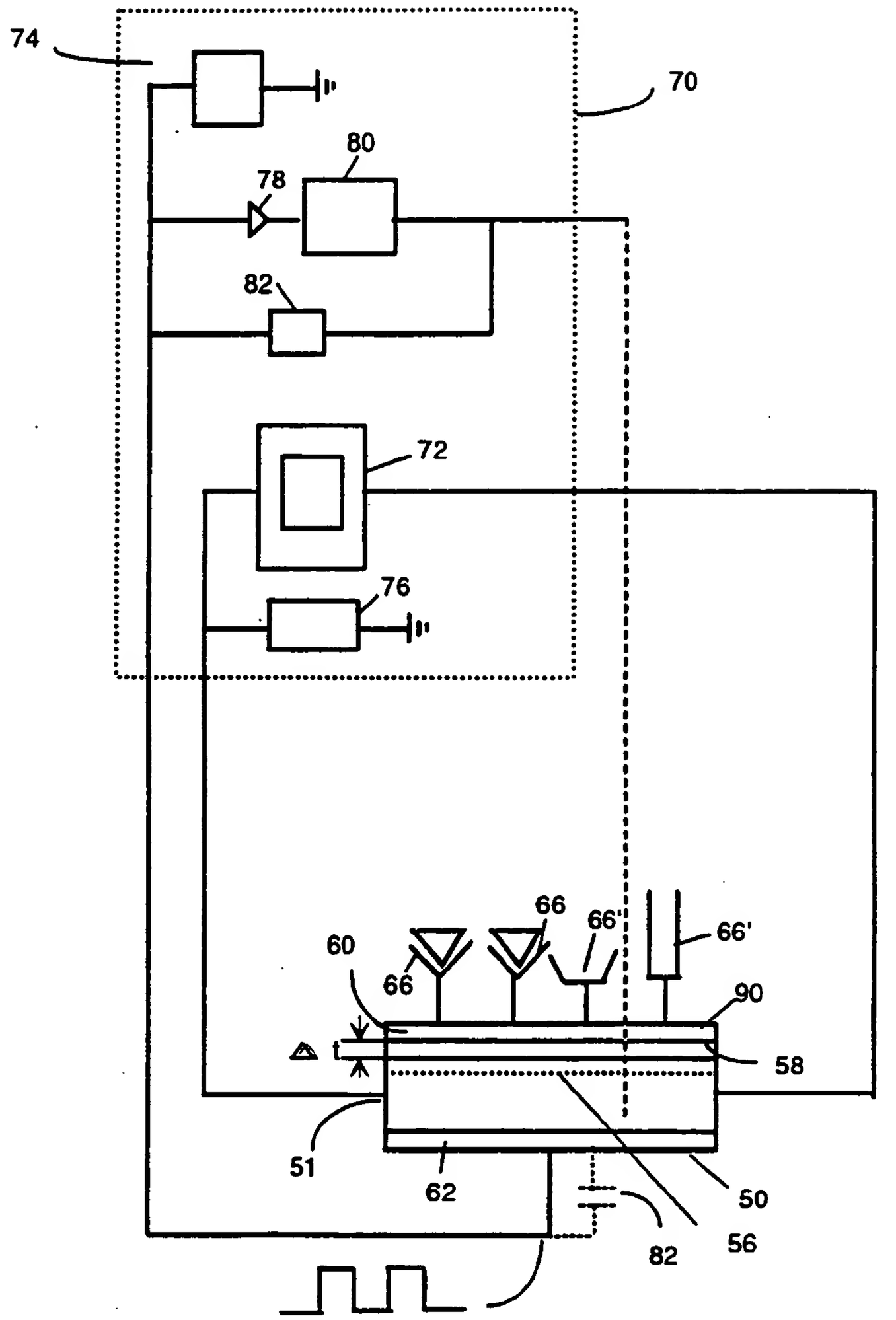

FIGURE 3B

(5/8)

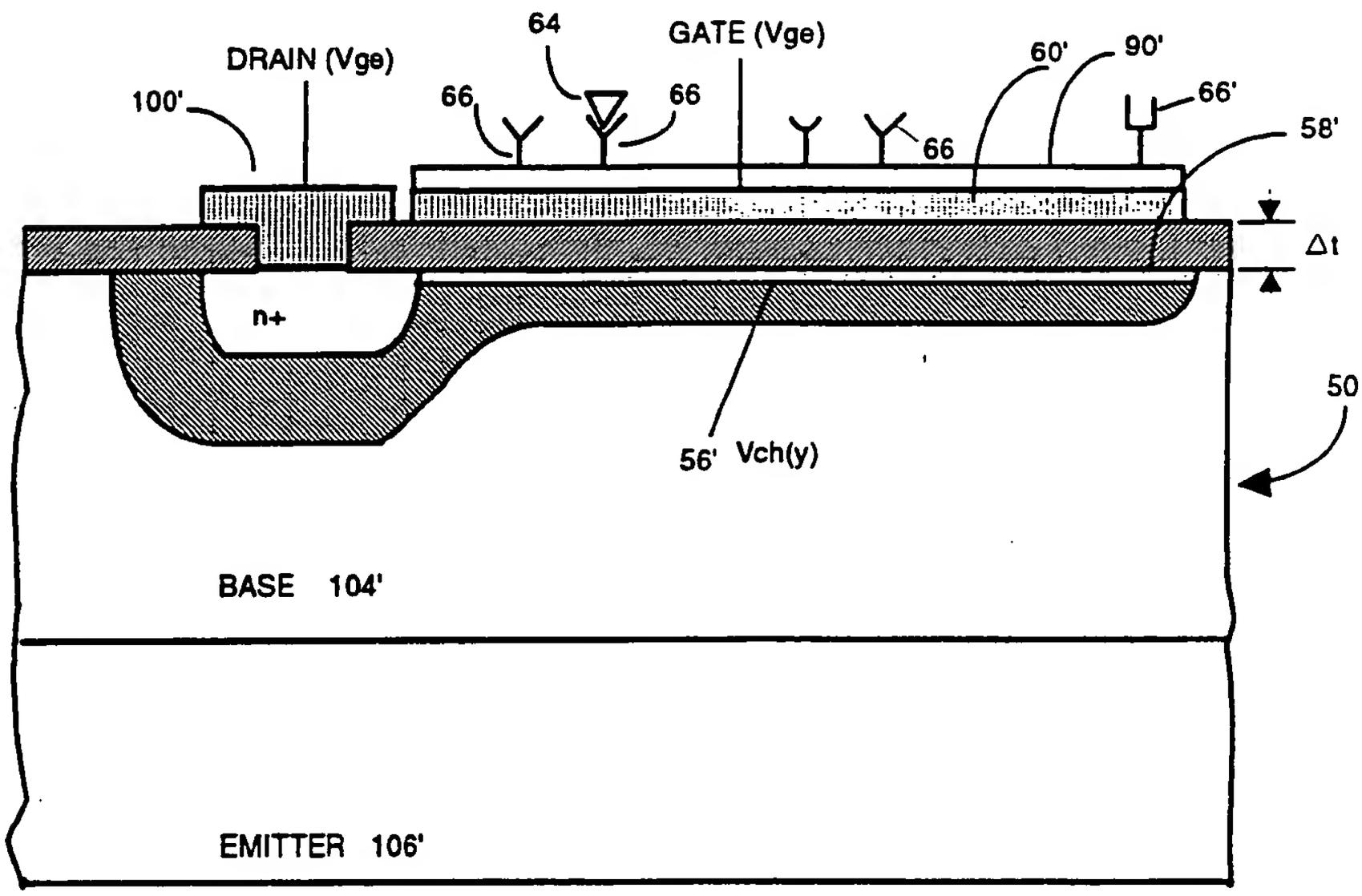

FIGURE 4

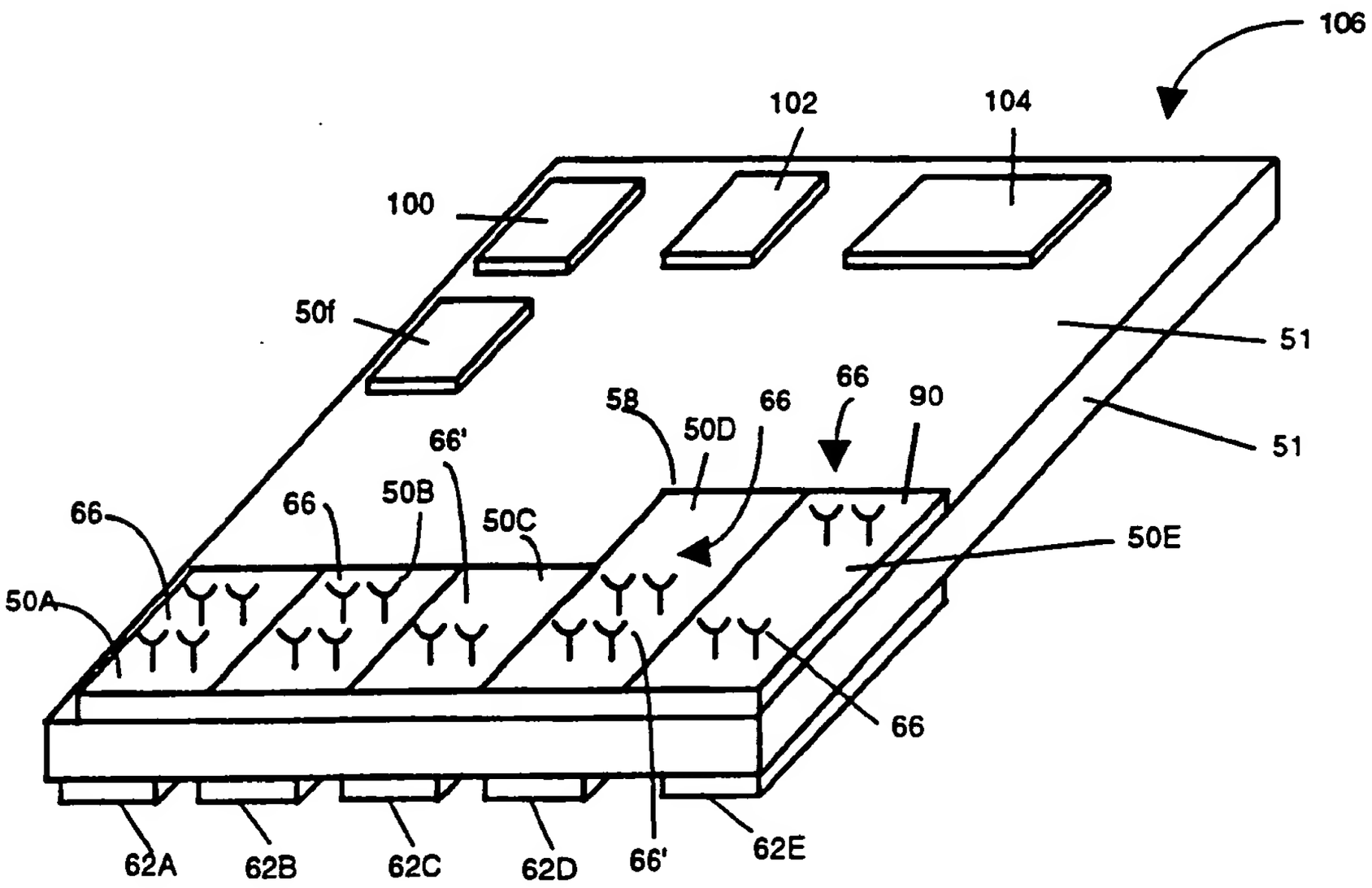

FIGURE 5

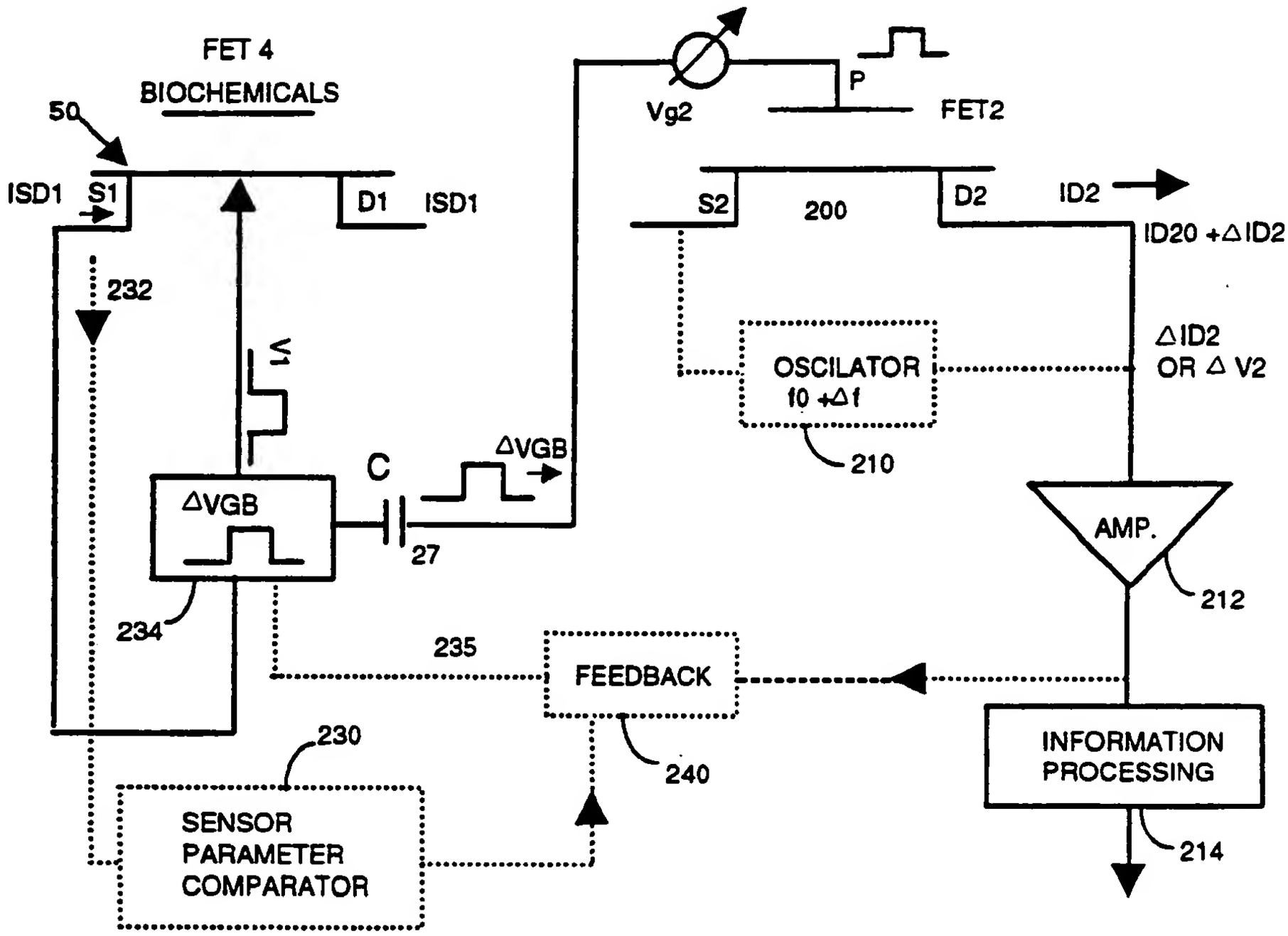

FIGURE 6A

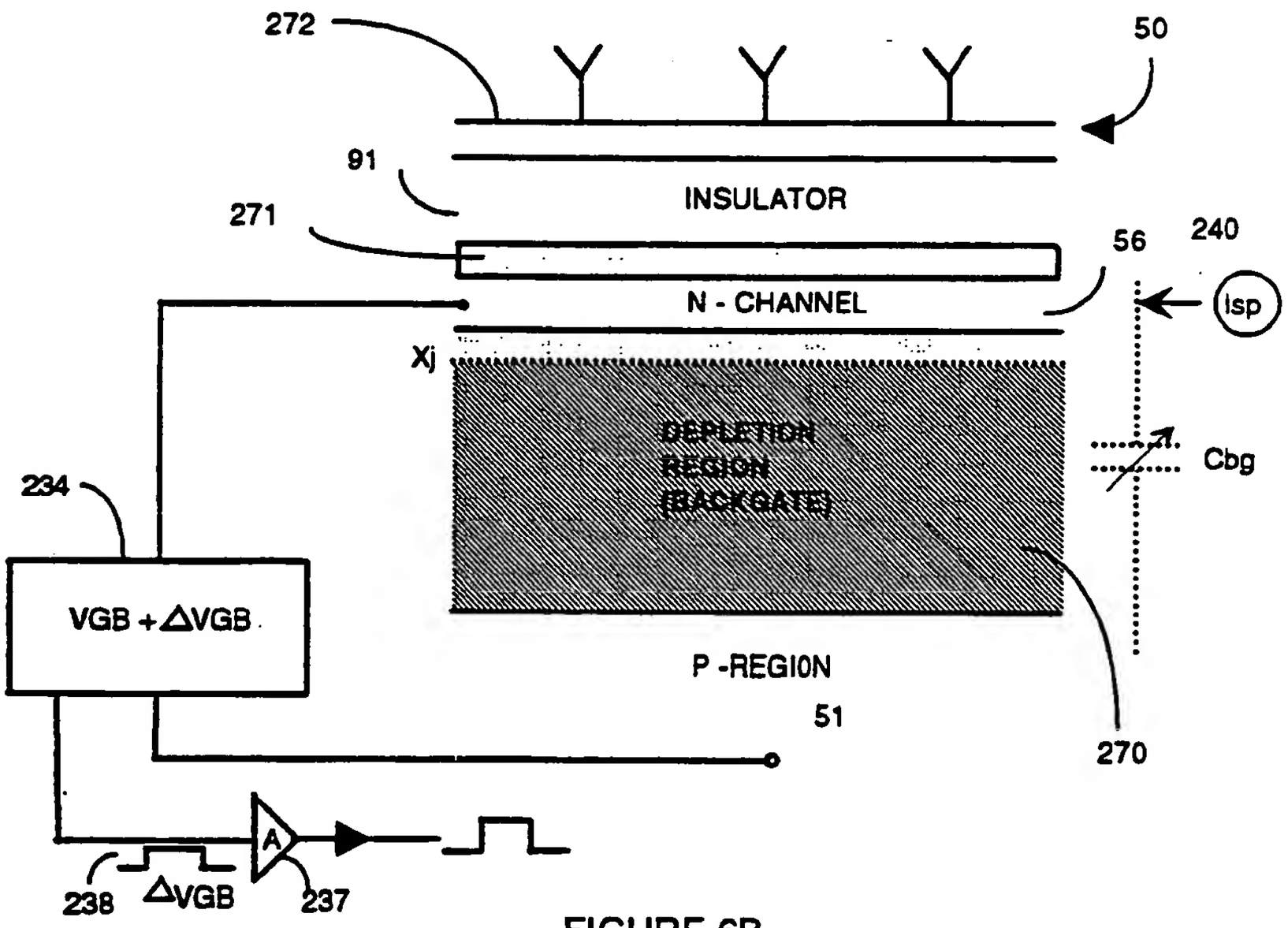

FIGURE 6B

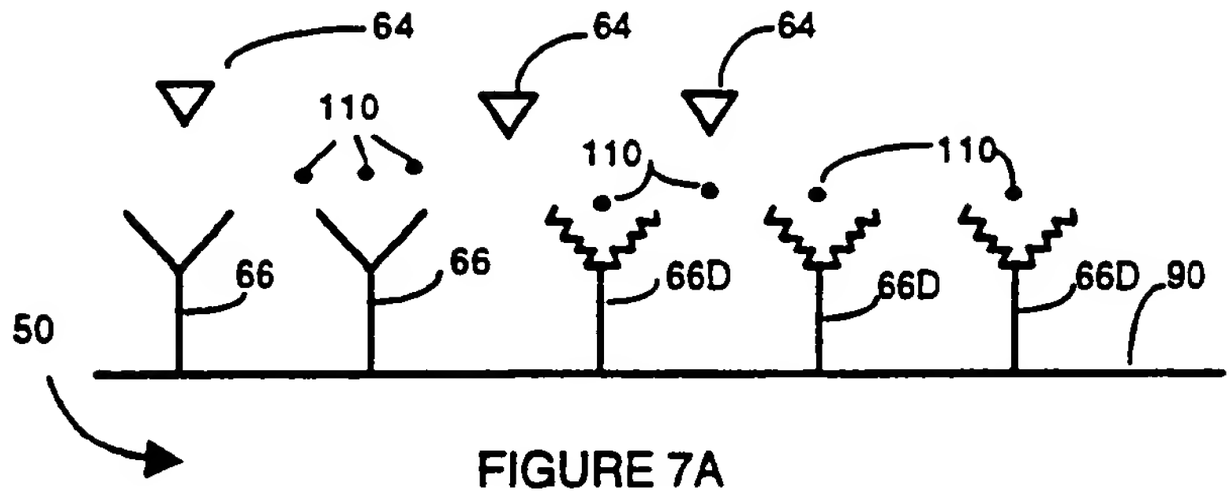

FIGURE 7A

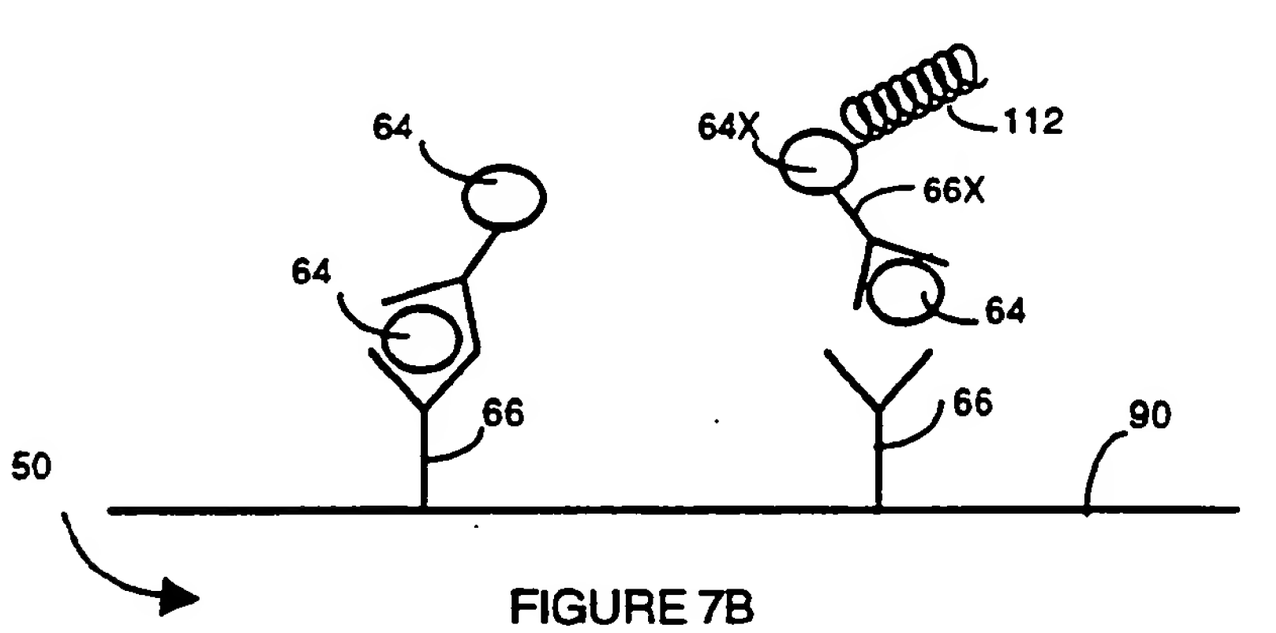

FIGURE 7B

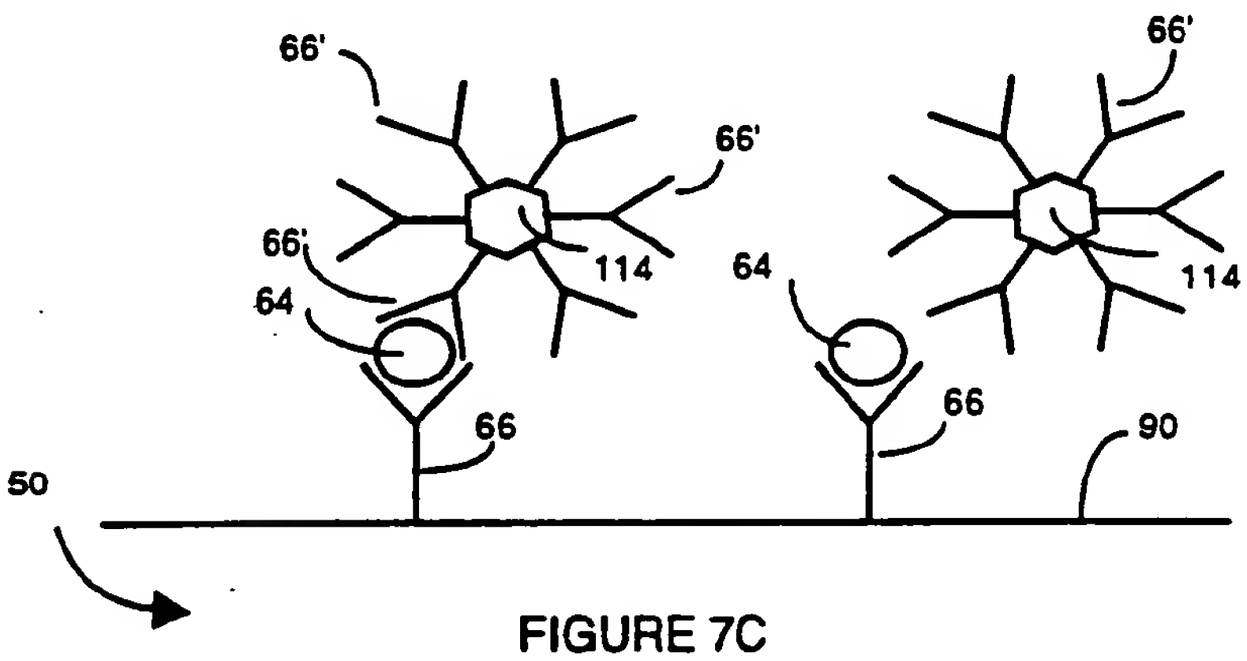

FIGURE 7C

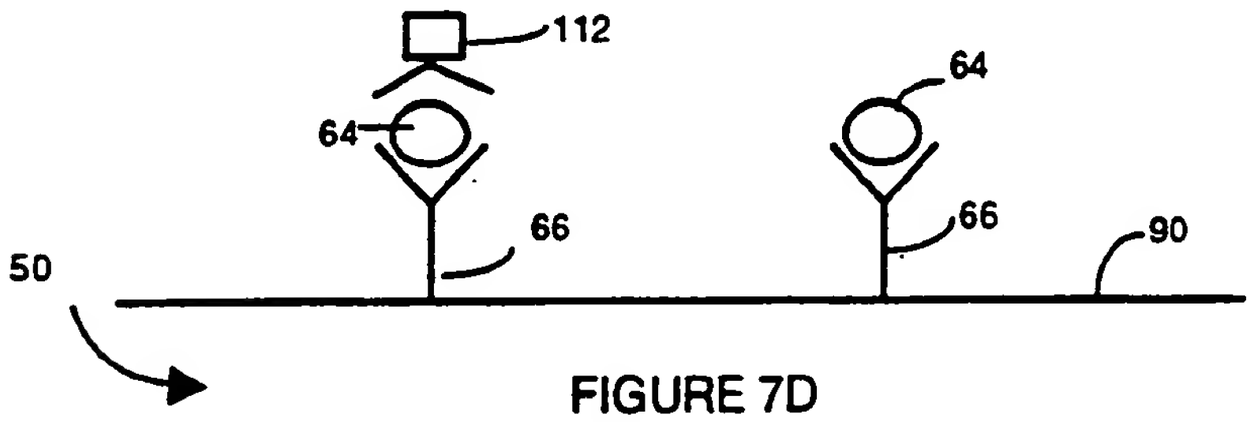

FIGURE 7D

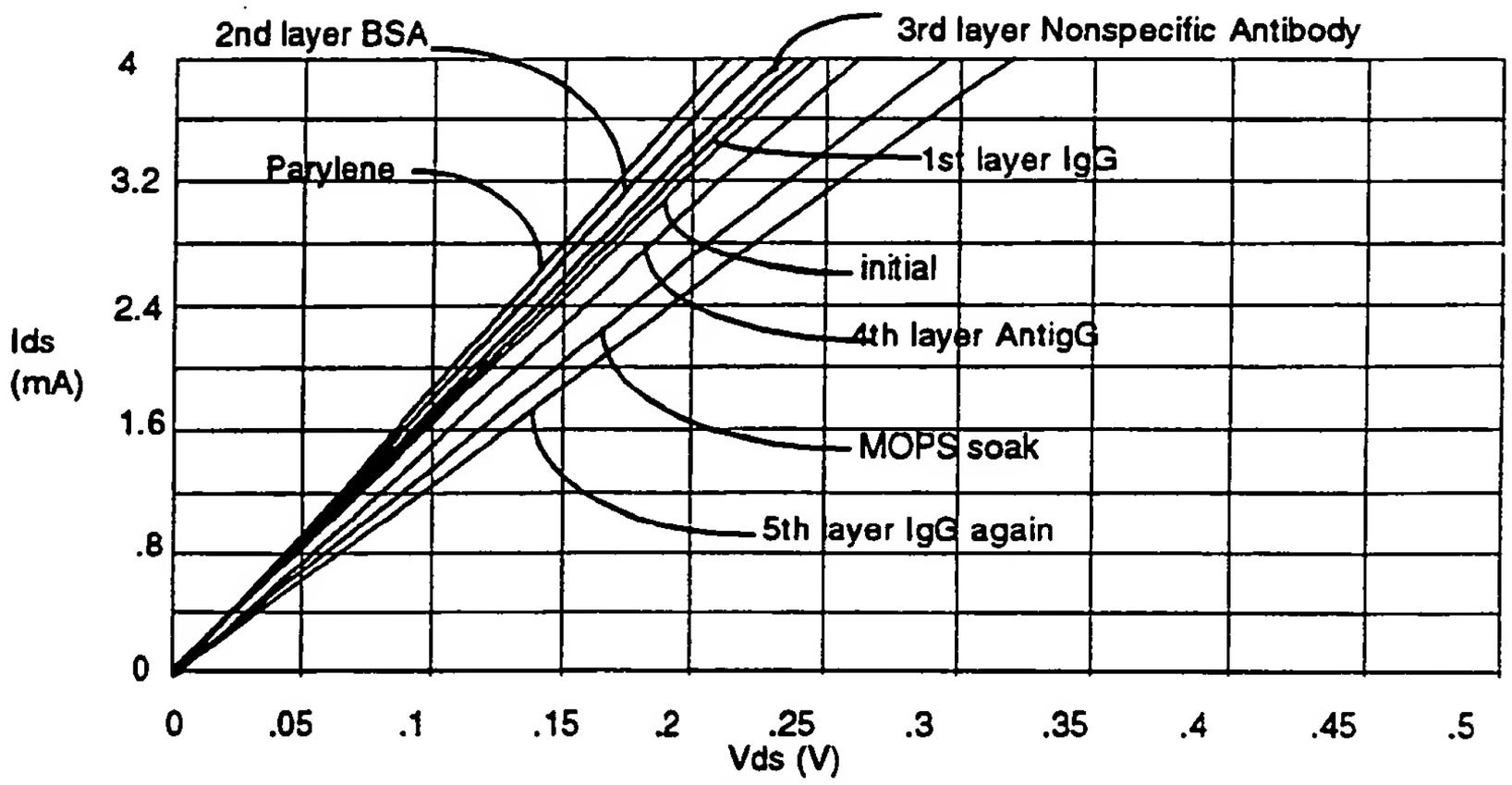

FIGURE 8A

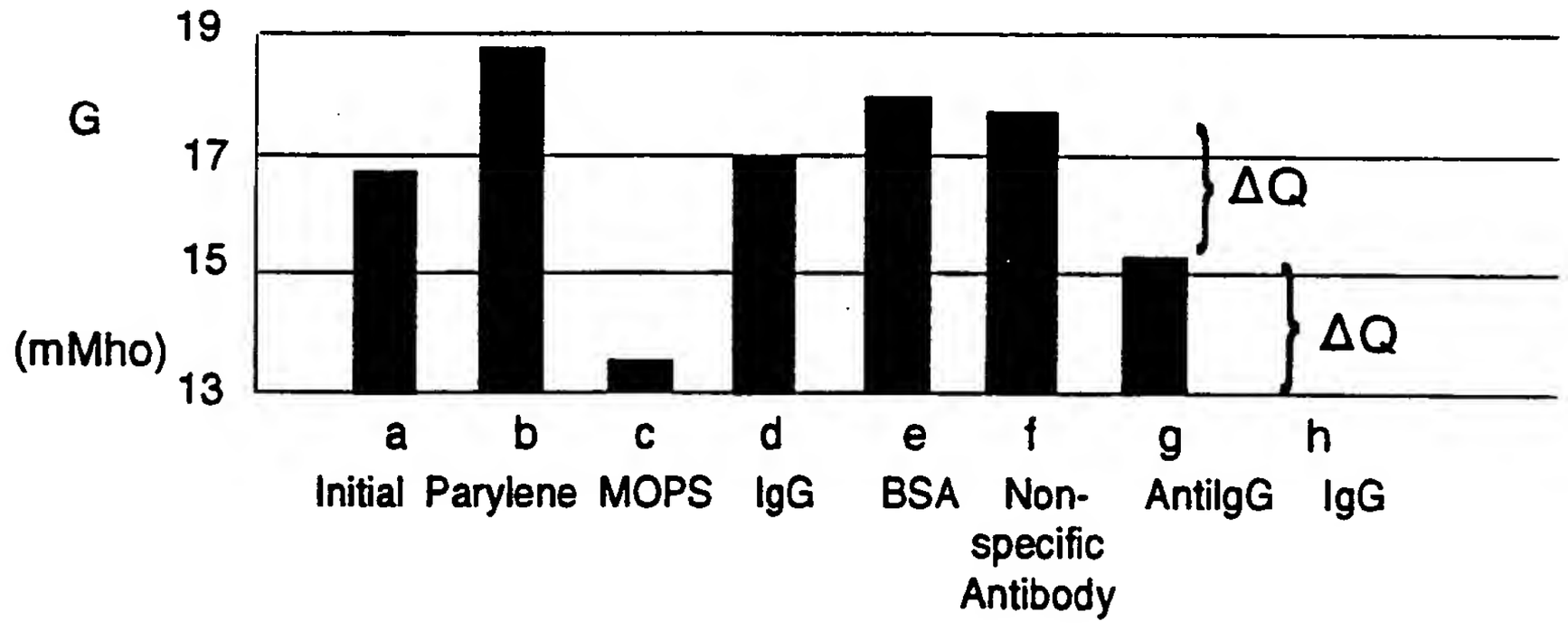

FIGURE 8B

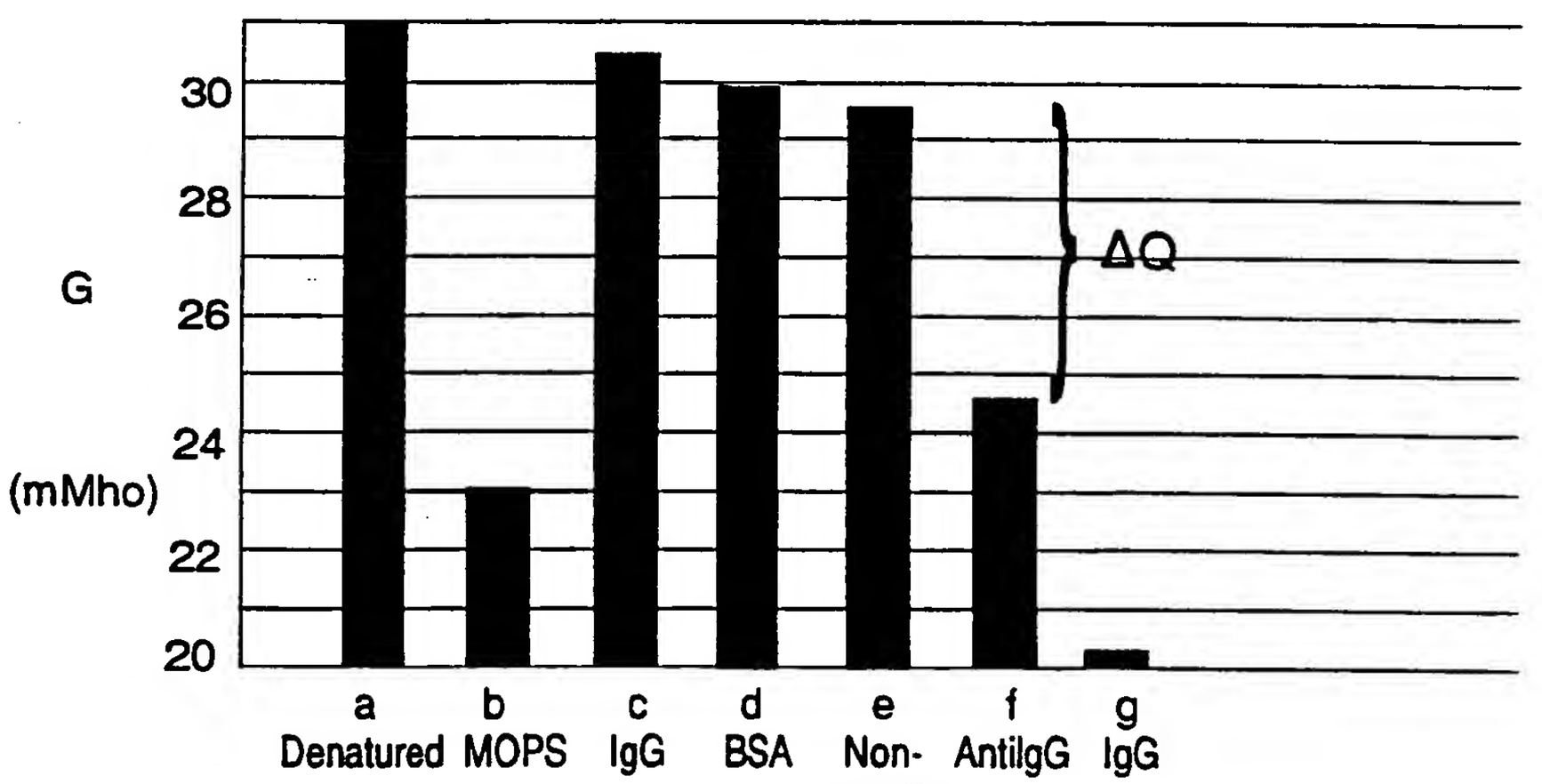

FIGURE 8C

INTERNATIONAL SEARCH REPORT

PCT/US92/08940

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :G01N 27/26; H01L 31/00
US CL :204/403,416,153.12; 257/253

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 204/403,416,153.12; 257/253

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,961,833 (SAKAI ET AL) 09 October 1990 (See column 3, lines 65-68 and column 4, lines 1-32)	1-6
Y	US, A, 4,180,771 (GUCKEL) 25 DECEMBER 1979, (See Abstract, column 3, lines 51-68 column 4, lines 27-68 and column 5, lines 1-2	1-10

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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Date of the actual completion of the international search

30 NOVEMBER 1992

Date of mailing of the international search report

13 JAN 1993

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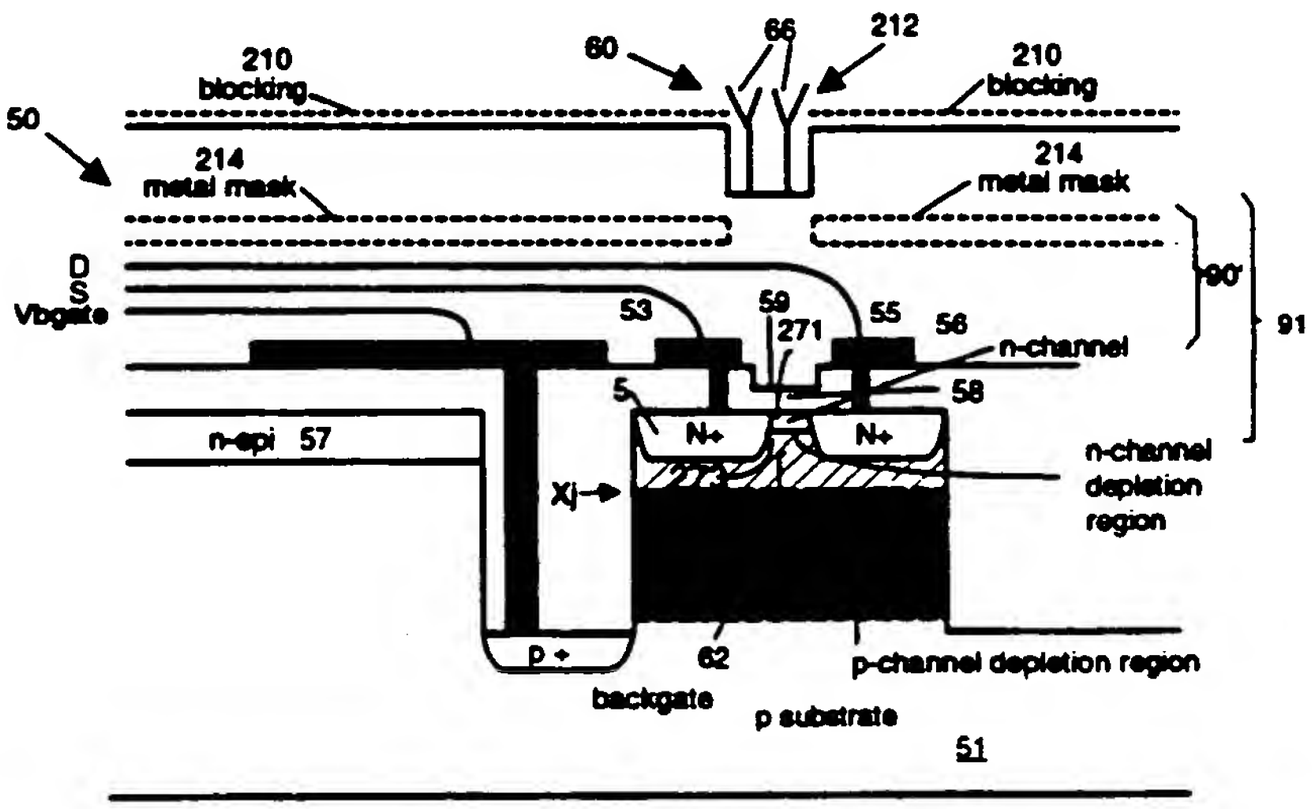
Published
With international search report.
With amended claims and statement.

(54) Title: METHOD AND DEVICE FOR BIOCHEMICAL SENSING



(57) Abstract

A method and application for detecting and measuring the presence of a binding target material (64) employs a semiconductor device (50) having a receptor-covered surface (90) topgate (60), separated by a dielectric layer (58) from a substrate (56). Receptors (66) attached to this surface exhibit a chemical selectivity function. Binding occurs in a test solution (52), with charge associated with the target material (64) modulating at least one device characteristic. According to the present invention, measurement may occur under dry conditions, at a time and location different from when binding occurred, thus substantially eliminating problems associated with ionic shielding and reference electrodes, so prevalent with prior art wet measurement techniques. Preferably the device (50) includes backgate (62) to which a bias may be applied to restore the device's pre-binding characteristics. Measurement of the restorative backgate bias provides a signal indicating binding of the desired target material.

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METHOD AND DEVICE FOR BIOCHEMICAL SENSING

RELATIONSHIP TO EARLIER FILED APPLICATION

This application claims priority from United States patent application serial number 07/781,479, filed on October 21, 1991.

FIELD OF THE INVENTION

This invention relates to sensing and measuring chemicals in general, and more particularly to methods and apparatus for sensing and measuring chemicals, biochemicals, molecules and sub-molecular components including ions, using semiconductor sensors.

BACKGROUND OF THE INVENTION

Semiconductor sensors for detecting biochemical reactions are known in the art, as exemplified by U.S. patent no. 4,180,771 to Guckel (1979). Figure 1 depicts a typical such prior art sensor 2 used to measure the attachment to a solid substrate surface 4 of a desired chemical compound 6 in a solution 8.

Sensor 2 typically is fabricated like a metal oxide silicon ("MOS") field effect transistor, where-in region 10 functions like a channel between source and drain regions 12, 14, and region 16 functions like a gate, but without metalization. Using receptor-type mechanisms 18, region 4 is made sensitive to (and encourages adhesion or attraction with) a desired target substance 6. Alternatively, receptor-like mechanisms 18 may be attached to the device gate 16.

Although region 4 should be relatively insensitive and non-reactive to other chemicals, such as 20, but in practice region 4 can respond non-specifically and attach with other than desired target substance 6. For example, solution 8 may also include charged particles 22 of varying size, including positive and negative ions. In biochemical sensing applications, a suitable biochemical environment for the receptor 18 and bio-target 6 dictates that test solution 8 have a relatively large ionic concentration. Unfortunately, relatively large ion concentration for the test solution 8 can adversely affect biochemical measurement due to ionic shielding.

Prior art measurements use a reference electrode to obtain stable and reproducible measurements, which electrode may be attached to sensor 2, e.g., electrode 24, or not attached, e.g., electrode 24' (shown in phantom). The reference electrode is coupled to a reference potential V_r (e.g., ground) and completes an electrical circuit, apparently to provide proper sensor biasing and to eliminate drift. Various bias potentials V_{sr}, V_{gr} and V_{dr} are coupled to the sensor, typically referenced to V_r. One or more measuring devices, indicated generically by 26, are also coupled to the sensor 2.

If the target substance 6 is present in solution 8, it should attach or bind to receptor 18, bringing electrical charges associated with the target substance. Target 6 attachment also brings mass to receptor 18, and can alter receptor 18's contact potential as well.

Thus, during binding or attachment, these electrical charges associated with receptor 18 influence charge present at region 4 (or gate 16, alternatively) and can measurably alter device 2's substrate bias, which can affect device 2's operating characteristics, including conductance and threshold voltage. By monitoring sensor 2 with detection and measurement equipment 26, these characteristic changes may be detected, indicating a binding of the target substance 6.

Further, charges at region 4 can also manifest a contact potential that tends to vary somewhat logarithmically with the charge concentration, a phenomenon sometimes used in sensing pH. It is characteristic of the prior art that measurements are made when binding of the target substance occurs, e.g., while sensor 2 is still immersed in solution 8.

Unfortunately such prior art sensors and sensing techniques have several deficiencies, including the use of reference electrodes, the inability to meaningfully directly measure charged particles including biochemicals (especially where the test solution is rich in ions), relative device insensitivity and drift, relatively high sensor production cost, and the perceived necessity to make "wet" measurements, i.e., while the sensor is in solution.

Prior art device reference electrode 24 or 24' unfortunately can contaminate the solution 8, and corrupt measurements. Further, the reference electrode bias V_r can interact unfavorably with any ions 22 present in the solution, resulting in ionic charge separation and polarization. Because even minute movement or agitation of solution 8 circulates these ions, potential disturbances are created that can affect measurement accuracy.

Further, sensing devices and procedures such as depicted in Figure 1 do not provide meaningful detection and direct measurement of charged particles, especially such particles exceeding a few angstrom in size, where the test solution has high ion concentration. In some

applications, the target to be detected is a charged particle 28 that may be several tens of angstroms or greater in size.

Unfortunately in Figure 1, ions 22, 30 in solution 8 can screen out and thus mask or shield the target charged particles. Thus, charges associated with the receptors and/or targets may be neutralized (in whole or part), thus masking the desired attachment signal.

To better appreciate the adverse effects of ionic shielding, assume that receptor 18 in Figure 1 has been charged positively (e.g., as a result of pH buffering of the solution 8), and that target material 6 is not yet introduced into the solution. Since solution 8 may includes ions 28, 30 of either polarity, mobile negative ions (assume 30) are attracted to receptor 18, and mobile positive ions (assume 28) are repelled. The polarized negative ions 30 shield or nullify the receptor 18 charge, causing a net charge of zero to be seen somewhat below the substrate surface 4. At the interface between the receptors 18 and substrate surface 4 the electric field is substantially zero, and thus the underlying FET is not influenced.

When added to the solution, target material 6 binds selectively to the mating receptor 18. But any material 6 charge experiences shielding due to ions in the solution, and produces net zero charge somewhat below the substrate surface 4, as indicated by the associated electric field. Thus, although a charged target material 6 has bound to the receptor 18, shielding prevents meaningful detection by device 2. Device 2's failure to sense attachment is a chronic problem with prior art devices, and may result in a false negative report. But to support certain medical and biochemical reactions of interest (e.g., many antibody-antigen reactions), the solution must have a relatively high ionic concentration that can result in a shielding length substantially masking, reducing or interfering with detection of the binding-charge induced signal of interest.

This apparent resultant low sensitivity associated with prior art FET type sensors (e.g., sensor 2) has caused such devices to be disfavored as sensors for the direct detection in solution of charged molecules, especially biochemicals.

Prior art sensor insensitivity is especially troublesome where relatively small changes (ΔS) in a signal (S) are to be measured. Rather than being able to provide a direct measurement of ΔS, such prior art devices sense $\log(S + \Delta S)$ and provide a signal proportional to $\log(S + \Delta S) - \log(S)$, at best a relatively insensitive indirect measurement of ΔS. Logarithmic dependent measurements are believed to account for the low sensitivity of typical prior art pH sensors.

Ionic shielding is not the only disadvantage with prior art in-solution sensor measurements. Wet testing can subject the measurements to drift resulting, for example, from ion movement within the solution, and from reference electrode contamination.

Further, in a given application the measurement and detection equipment 26 may require sophisticated and expensive components. Under such circumstance, having to "wet test" requires that the test and detection/measurements occur essentially at the same time and place as the target binding. This restriction can preclude the use of sensors if sophisticated equipment is not readily available in the region where the testing (that possibly leads to binding) occurs.

It would be advantageous if after possible binding, the sensor could be sent, preferably dry, to a remote facility for detection and measurement of any target substance attachment using sophisticated equipment not available at the testing/binding region. Unfortunately, such "dry testing" is not practiced with prior art devices and procedures such as depicted in Figure 1.

Fabricating many prior art sensor devices is sufficiently expensive as to preclude "use once and discard" practice. Similarly, often the receptor material is scarce or very expensive. Clearly it would be advantageous if devices and/or their receptor materials could be used more than once. In addition, sufficiently inexpensively fabricated devices could be provided in arrays to permit simultaneous testing, included automated testing for multiple target materials simultaneously (e.g., multiple disease antigens), included automated testing.

Many prior art sensors have limited sensitivity, limited sensor gain, and/or device drift, unfortunate limitations since in many clinical applications, a target biological analyte may exist in a minute concentration, i.e., a few ng/ml for proteins in blood serum. Substantially more sensitive devices would permit the simultaneous use of several different dedicated receptors to provide more rapid (and thus less expensive) testing, including differential analysis testing.

In short, there is a need for an inexpensive immunosensor, preferably a IC-compatible (preferably integrated with signal enhancing, control and other environmental sensors, all on-chip), FET-type device that can be inexpensively mass produced using standard semiconductor fabrication technology. Such device should reliably measure biochemical information with high sensitivity, and be substantially free of signal drift.

Further such devices should include multiple receptors, some of which may be dedicated to binding different target materials, and should further include a mechanism for discerning which of several target materials have in fact bound. Further, there is a need for devices that may be fabricated and used in arrays, including arrays containing sensors with multiple types of

receptors. Such arrays would promote rapid and relatively inexpensive testing, including differential and confirmational analysis testing, including self-testing of the devices themselves, and testing that environmental feature such as temperature and pH are appropriate to insure that valid testing has occurred.

Preferably such device, and a methodology using such device, should not require a reference electrode, and should be capable of making measurements under wet or dry conditions. Further such device and method should enable detection of a contact potential resulting from the binding of a target material and a receptor, and should include mechanisms to eliminate false positive and false negative measurements resulting from non-unique pH_{oc} values.

Further, such device and methodology should provide mechanisms for enhancing the sensitivity of the device per se, for enhancing the effective amount of charge binding to the device, and for amplifying the signal detected by the device. Preferably such mechanisms should be usable and reusable under wet or dry measurement conditions.

Finally, such device and methodology should be useful in a wide spectrum of applications including biochemical sensing and measurement, DNA research, pH and hydrogen sensing, pollution sensing, optical and photodetector sensing, pyroelectric sensing, magnetic and force sensing including piezoelectric sensing.
The present invention provides such devices and methodologies.

SUMMARY OF THE INVENTION

In a first aspect, the present invention provides a field effect type device that can be used for wet or dry detection and measurement of a binding target material, without requiring a reference electrode. In a first embodiment, the device includes a semiconductor material whose upper surface is covered by an insulator layer supporting a top gate, and a lower surface that supports a bottom gate. When coupled to a power source, the device creates a field effect region, and includes a channel capable of conduction as a function of electrical activity at the top gate and/or bottom gate. The top gate preferably is covered by a binding layer that selectively provides a chemical reaction function in the presence of a predetermined class of target, such as charged particles, bio-particles, chemicals, etc. In a second embodiment, a distributed channel bipolar device having bipolar and MOS device characteristics is employed as a sensor.

Such devices may be implemented in a variety of ways using field effect device phenomena, such associated with a junction field effect transistor ("FET"), an exposed insulator FET ("EIFET"), a non-metalized gate metal-oxide-silicon field effect transistor ("MOSFET"), a non-metalized gate metal-insulator field effect transistor ("MISFET"), a heterojunction device, and

a field effect capacitor. Further, the field effect region of the device may be operated in a variety of modes, such as enhancement, depletion, inversion or accumulation. In the first embodiment, the bottom gate may be implemented in several ways, including as a PN junction, a PIN region, and an inversion channel/substrate. Preferably the bottom gate structure is relatively lightly doped such that a voltage applied thereto is dropped primarily away from the channel, to enhance sensitivity of the device.

In use, the device is exposed to a solution containing a target material with which the binding layer will attach. If attachment occurs, the resultant change in attachment charge and/or contact potential will alter the device's quiescent state. Such induced charge effects may be used with a FET-type sensor statically, sequentially, or transiently. Suitable monitoring equipment coupled to the device can detect this change, confirming that the target substance is indeed present, and providing an quantitative measurement. Various amplifying means and feedback features may be to enhance sensitivity and performance.

In a second aspect, the present invention provides measurement of the device to occur in a dry, or at least quasi-dry state. Measurements may also be made in the conventional wet state. In dry/quasi-dry testing, the device is exposed to a test solution wherein binding with a target substance may occur. The device is then dried such that attached or bound target substance remains attached to the device. The device is then measured dry (or re-wetted), at a later time and different location, if desired.

The present invention's ability to dry measure avoids the ionic shielding problems and resultant low sensitivity associated with prior art wet measurement techniques. As a result, the present invention permits meaningful direct measurements of charged matter, including particles, ions, many biochemicals, nucleic acid chains and components such as DNA and DNA segments, and so forth.

In further contrast to the prior art, the present invention requires no reference electrode, and thus avoids reference electrode contamination of the test solution, signal drift, and ionic disturbances. Mechanisms are disclosed for selectively attaching receptors to desired regions of the sensor, to enhance performance and to protect any other components on the substrate from deleterious attachment effects.

In yet another aspect, the present invention provides various mechanisms, suitable for wet or dry measurements, for enhancing the attachment of target material to the device, for enhancing sensitivity of the device to attached target material, for providing confirmational data including confirmation that the devices themselves are functioning, and for processing signals from the device representing attachment.

Other features and advantages of the invention will appear from the following figures and from the following description, wherein the preferred embodiments are set forth in detail.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 depicts a generalized sensor and sensor measurement, according to the prior art;

FIGURE 2A is a perspective depiction of an EIFET sensor, according to the present invention;

FIGURE 2B is a side view of an EIFET sensor, according to the present invention;

FIGURE 3A depicts a sensor and generalized sensor measurement according to a first embodiment of the present invention;

FIGURE 3B depicts a sensor and generalized sensor measurement according to a second embodiment of the present invention;

FIGURE 4 depicts an alternative embodiment for a sensor, using a distributed channel bipolar device;

FIGURE 5 depicts an embodiment for a sensor array that includes sensors having multiple receptors including neutral receptors, and sensors with on-chip memory;

FIGURE 6A depicts a cascode arrangement for signal enhancement, according to the present invention;

FIGURE 6B depicts an embodiment wherein device current is constant and back bias is modulated and sensed;

FIGURE 7A depicts sequential use of a blocking agent, according to the present invention;

FIGURE 7B depicts the use of sandwich-like structures and conglomerates to enhance detection, according to the present invention;

FIGURE 7C depicts the use of beads to enhance detection, according to the present invention;

FIGURE 7D depicts the use of conjugates to enhance detection, according to the present invention;

FIGURE 8A depicts changes in the characteristics of a device according to the present invention, in response to different attachment phenomena;

FIGURE 8B is a bar graph conductivity depiction of the slope of the data shown in FIGURE 7A;

FIGURE 8C is a bar graph depiction of conductivity change in a device according to the present invention, in response to exposing the device to various solutions including a denaturing solution.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Figures 2A and 2B depict a sensor 50 implemented as a semiconductor field effect device, such as an FET. Device 50 may be fabricated in a variety of configurations, including a junction FET ("JFET"), a metal-oxide-silicon FET ("MOSFET"), a metal-insulator-silicon FET ("MISFET"), an exposed insulator FET ("EIFET"), an ion sensitive FET ("ISFET"), a distributed channel bipolar device ("DCBD"), a heterojunction device and a capacitor. Further, semiconductor device 50 may be operated in enhancement mode, depletion mode, or inversion mode. It is significant to note that no reference electrode is depicted or required according to the present invention.

Device 50 includes a semiconductor substrate bulk 51 having a source 53, a drain 55, an FET channel 56 capable of electrical conduction, an insulation layer 58 such as SiO_2, Si_3N_4, etc., an exposed insulator topgate 60, and a bottom or lower gate 62. According to the present invention, the conductivity of channel 56 may be modulated by electrical signals and/or charge present at topgate 60 and/or bottom gate 62. Preferably device 50 is an exposed insulator FET, or "EIFET", with a relatively deep channel 56 (under no backgate bias) to increase device detection sensitivity.

The uppermost surface 59 of insulator layer 58 is preferably covered with a protective moisture blocking film 90 that is relatively inert chemically, impervious to any corrosive components in solution 52, will not contaminate the test solution, and provides an outer surface with many sites for binding with target specific receptor substances 66. Applicant has found parlyene to function especially well as film 90, although other materials could also be used. Further, this structure allows the topgate to float at the potential of the test solution, thus avoiding a topgate bias that could influence the binding reactions at the topgate and introduce erroneous signals. As a result, the surface of film 90 can be made selectively reactive without contamination risk.

Applicant's depletion mode EIFET device (Figures 2A and 2B) had a 92.5 nm thickness for layer 58, an approximately 100 nm parylene film layer 90, an n-epitaxial layer channel 56 with

4.6 microns thickness, doped at about 10^{15}/cc (calculated to be 3.4 microns absent back bias), with W/L ~ 1432 and source-drain distance L = 20 microns. As shown by Figure 2A, the gate 60 was fabricated with a meanderline pattern to provide a large W/L using the laboratory equipment at hand, to thus enhance device sensitivity. In Figure 2A, backgate 62 is the pn junction between substrate 51 (p material) and channel 56 (n-epi region 57). Of course other polarity dopants could be used.

It was not feasible to fully deplete through the device channel due to leakage current generation problems associated with the prototype. However, even with partial depletion of the epi channel, large receptor and target attachment signals were observed. These signals would be even larger for the near total channel depletion condition, 1 μ drain-source dimensions (thus increasing W/L) that a commercially fabricated device should produce. A commercially fabricated device would further permit very high backgate amplification, with further sensitivity enhancement.

According to the present invention, attached charge influences an underlying channel region in a measurable fashion. It is therefore desired that receptors 66 be affixed to the present invention 50 over the topgate region 60, but not elsewhere. In a conventional integrated circuit, the overlying field oxide layer is of no use in protecting underlying regions against attached surface charge. With reference to Figure 2B, selective receptor attachment can occur in several ways. In a first method, the uppermost surface of device 50 is masked with an inert agent or blocking agent 210 that blocks attachment to material 66 such that only the region 212 over the topgate 60 remains unmasked and thus receptive. The device is then exposed to a solution containing the desired receptor material 66, which attaches over the topgate region 212, but not elsewhere due to the blocking material mask 210.

Alternatively, mask 210 may be a preselected blocking chemical with a predetermined charge polarity, chosen such that underlying regions (e.g., devices or circuits) are not unduly influenced by charge attached to mask surface 210. Where a specific masking chemical layer 210 has been patterned to protect underlying regions, post target attachment treatment can be used, such as preparation with a buffered pH solution corresponding to the layer 210's isoelectronic point.

In a second method, the device includes metalization patterns 214 that preferably shield all of the device save for the desired topgate region 212. Preferably the metal mask 214 is covered by a layer of a material 90, e.g. parylene, to minimize possible test solution contamination by the mask. The device is then exposed to a solution containing the desired receptor material 66. Receptors 66 attaching over the topgate region 212 will be in field communication with the underlying channel region 56, whereas receptors attaching elsewhere will have their charge

(and indeed any subsequently attached target material charge) shielded from influencing regions outside of the topgate region 212 by the metal mask 214, which is coupled to a DC potential or ground.

The potential is selected so underlying regions are not unduly influenced by charge carrying chemicals attached above the shield region 214. Thus, shielding, including buried metal masking, is especially desired where the substrate 51 includes other components, such as depicted in Figure 5. But for the desired shielding action provided by the mask 214, performance of other components (e.g., 100, 102, 104) disposed beneath receptor material 66 could undesirably be influenced by the resultant electric field changes produced by the receptors and/or any targets attached thereto.

A selected primary receptor 66 is selectively attached to the outer surface of film 90, during or after fabrication of device 50. Essentially primary receptor 66 dedicates device 50 to bindingly detecting a mating target substance 64, but not other substances 68, 70, 72 that may be present in the solution (see Figures 3A, 3B), which other substances may include charged materials and positive and negative ions. As depicted in Figures 5A and 5B, device 50 may in fact include two or more receptor types 66, 66', each of which is dedicated to bind with a different target material.

Alternatively, where simultaneous testing for multiple target materials is desired, an array of sensors incorporating one or more specific receptors, may be used (see Figure 5). Such sensor arrays may be prepared for sensing multiple target substances using photo-patterning known to those skilled in the relevant art. An additional advantage of an embodiment such as Figure 5 is that degradation of device sensitivity due to long lead and contact resistance can be reduced by integrating associated circuitry (100, 102, 104) on a common substrate with on-chip interconnects. Such on-chip interconnects further minimize test solution contamination associated with lead contacts and electrodes.

While generally the particle or, target substance, 64 will be a chemical or biochemical, the present invention can also test for the presence of photons, the effects of force, magnetic fields, electric fields, and the like. As used herein, "chemicals" includes not only solutions, molecules, ions, and atoms, but also subatomic particles, such as electrons. As used herein, "biochemicals" includes not only biochemical compounds such as sugars, fats, proteins, etc. but also polymers such as proteins, nucleic acids, glycosaminoglycans, and the like, and encompasses microorganisms and fragments thereof, such as bacteria, viruses, and protozoa.

With reference to Figure 2B, preferably, applicant's depletion mode EIFET structure includes a lightly doped n-epi layer 57 (e.g., the built-in channel) fabricated on a low resistivity p substrate 51, wherein N+ regions 53, 55 form the device source and drain regions. The gate 60 structure is somewhat similar to a JFET with a MOS gate, but without metalization. Like a JFET, the device has a built-in channel 56 that, operating in depletion mode, is normally on, with the depth of the built-in channel (and thus channel conductance) controllable via a reverse bias coupled between source 53 and substrate 51.

While the present device 50 provides good sensitivity, sensitivity enhancement strcutres can provide yet additional sensitivity. Typically the topgate bias causes depletion or accumulation of the channel region 56 immediately under the topgate 60. (Alternatively, if the device 50 incorporates an inversion channel, topgate bias influences the extent of the inversion, e.g., channel conductance.) The backgate is preferably reverse biased to permit the device to operate in a highly sensitive regime under pre-attachment conditions. A relatively small change in a gate attached charge modulates channel depletion width 56 and channel conductance. This condition also requires the largest restoration backgate potential 74, 234 (Figures 3A, 6A) to reset the device 50 to the pre-attachment condition.

With reference to Figures 3A and 6A, preferably a device according to the present invention uses backgate bias 74, 234 to restore device operating characteristics to a preattachment condition, where a device operating parameter is selected as a reference value. For example, the reference parameter can be source-drain current, pinch-off voltage, transconductance characterized by AC signals, source-drain voltage for constant source-drain current, etc.

In this operation mode, the device backgate 62, 270 is biased to restore the preattachment reference characteristic. Thus if attachment increases channel depletion 62, 271 in the depletion mode, a preattachment reverse backgate bias would require reduction to restore preattachment operating drain current, where source-drain voltage was held constant. The shift in backgate bias to restore the preattachment condition provides a measure of attachment. In depletion mode operation, the backgate voltage preferably almost pinches off the channel 56 before attachment of charge to the topgate. This bias regime permits the largest percentage change of channel resistance and current to be determined, and advantageously requires the largest restoring back bias voltage.

The use of a measurable backgate bias to a restore preattachment condition permits automation using circuits and components known to those skilled in the relevant art. For example, a voltage divider providing a controllable output voltage coupled to the backgate could be used with a simple feedback circuit 240 to reestablish drain-source current, the amount of restoration voltage providing a measure of the attachment condition.

A reference pulsed drain-source current 240 could also be maintained with a pulsed backgate voltage 234. The resultant pulsed current could be detected and used to adjust, via feedback, the pulsed backgate voltage to restore the current to the pre-attachment value. Alternatively, a pulsed AC drain-source current dependent upon device transconductance could be used. The use of AC signals such as time dependent backgate pulse voltages advantageously permits DC blocking capacitors 27 to be used, AC amplification 212 to be incorporated free from DC drift influences, and allowing AC filters to reject noise to enhance device sensitivity.

Sensitivity enhancement is attained because for PN junctions (e.g., a backgate pn junction 270, or a reverse biased channel-substrate 270), the amount of depletion resulting from a small reverse bias voltage change is generally dependent upon the initial reverse voltage magnitude. For example, for a one-sided abrupt junction whose n-side is doped at $10^{15}/cm^3$, the depletion width is approximately 5 μm at 20 V reverse bias, and is about 6 μm at 40 V reverse bias. It is this nonlinearity that may be used to increase measurement sensitivity in a device employing a suitable backgate.

Thus, substantially reverse biasing the backgate requires a relatively large incremental change in reverse bias (compared to an initial lower reverse bias potential) to provide a given change in depletion width necessary to reset or reestablish preattachment channel conductance. Where source-drain output current is to be maintained, the backgate potential is modulated to restore the conducting channel to a preattachment conductance. Whether an increase or decrease in backgate reverse bias 74, 234 is required will depend on whether attachment increases or decreases depletion or accumulation beneath the gate region 60. The magnitude of voltage change 74, 234 to restore preattachment channel condition increases with increasing initial backgate bias, thus enhancing device detection sensitivity to attachment.

If this incremental backgate bias change 74, 234 is then coupled to the gate of another FET device (FET 2), a much larger output signal 212 is available from FET 2 than would have initially been present in the first device 50 due to attachment induced conductance modulation effects. In this fashion, device attachment sensitivity is increased. If the backgate 62, 270 is operated in AC pulsed mode, AC amplification of the signal delivered to the second device can yet further enhance sensitivity.

Device 50 can be designed to enhance sensitivity by selection of dopant and doping profiles for the pn junction comprising the backgate region 62, 270. Where both regions are homogeneously doped, depletion extent will be the same on either side of the junction. If however one region 273 is more heavily doped than side 274, then 273's side side will experience a shallower depletion extent, depletion being inversely proportional to the homogeneous doping ratio. For example, if the junction's n side is 10^{+4} more heavily doped

than the p side, applying a given incremental reverse backgate bias results in about 10^{-4} of the incremental bias falling across, and modulating, the n region. Stated differently, a substantially larger incremental voltage must be applied to the backgate 62, 270 to restore a preattachment state than would be the case if the two sides of the junction were equally doped. In essence, the two sides of the pn junction perform a voltage division function, providing a small fraction of the total backgate bias change to the channel side of the backgate junction. Non-linear junction doping can also enhance this desirable sensitivity enhancement feature of a backgate, according to the present invention.

Other back gate junctions may be used with the present invention to enhance target detection sensitivity. A P-I-N backgate substantially enhances sensitivity using a large intrinsic region, and dropping a large fraction of applied backgate restoring potential across the I region. Thus, a very small fraction of the backgate bias modulates the n-channel width, leading to especially large sensitivity enhancement.

Figure 3A depicts a first preferred "wet" measurement embodiment wherein a device 50 contacts a test solution 52 within a container 54, such as a microtiter plate. In operation, film 90 and receptor 66 portion of device 50 are exposed to the test solution 52, allowing any target substance 64 present to bind or attach. Because substance 64 has an associated electrical charge, post-attachment electrical charges will be present at receptors 66 that can affect the performance of device 50.

As noted, semiconductor device 50 will generally include at least one field effect region wherein the conductivity of a channel 56 is modulated is response to electrical or charge activity on one or more gates 60, 62. Such charge (from bound target 64, for example) creates an electric field that operates through the thickness Δt of a typically dielectric insulating layer 58 (e.g., SiO_2) and terminates on charges within the semiconductor, thereby altering the semiconductor channel region 56. The magnitude and quantity of such bound charges alters the resistance of the conducting channel.

Alternatively, the device may not employ a conducting channel. A sensor field effect device could be implemented as a capacitor device that senses chemical associated charge attachment by modulating capacitance change, which change could be sensed by including the capacitor in an appropriate electronic circuit, an RC oscillator, for example.

For a field effect transistor ("FET") device, the resultant electric field modulation of the channel conductance (or resistance) alters the typically drain-source current flow in the channel. Charge binding thus alters one or more otherwise quiescent characteristics of device 50, which alteration may be sensed and measured, qualitatively and/or quantitatively, using equipment

70. The measurable characteristics may include, without limitation, drain current, change in threshold voltage, pinchoff voltage, gate-source voltage, transconductance, conductance, gate-source capacitance, gate to substrate capacitance, back gate capacitance, transconductance threshold voltage "DCBD", and gated bipolar devices), bipolar current gain (DCBD), transconductance (DCBD), and change in source voltage for constant drain current.

For example, the attachment of a target-originating charge sheet results in an associated electric field generated across a thickness of insulator material, which results in a threshold voltage shift in an insulator gate field effect device. The resultant incremental threshold voltage change alters the device's operating characteristics, which characteristics may be used to influence an associated circuit to provide an enhanced measurement signal representing attached charge. Such measurement may represent, for example, channel resistance, switching time, oscillator frequency (where the device is incorporated in an oscillator circuit), channel transconductance, output current into a bipolar transistor base, etc. Further details regarding field effect theory and modelling may be found in a number of standard treatises, including "Device Electronics for Integrated Circuits", 2nd ed., by Muller and Kamins, published by John Wiley.

In the configuration of Figure 3A, attachment measurements are made while device 50 is still in the solution 52, typically at the time of attachment, or in some other solution (here shown as 52 also) whose pH, temperature, chemical composition, etc. may differ from test solution 52. As shown, during measurement device 50 is coupled to measurement equipment 70, which preferably includes one or more measuring instruments 72 such as an oscilloscope, current probe, semiconductor parameter analyzer or curve tracer, capacitance measuring instrument, specially designed instruments sensitive to the sensor parameters of interest, and the like. Equipment 70 also includes a variable power source 74 that is preferably coupled to device 50's bottom gate 62. Optionally, equipment 70 can include an additional power source 76, coupled to device 50, for establishing a pre-binding quiescent state.

When a target substance 64 binds to a receptor 66, the associated substance charge and/or contact potential will alter device 50's conductance state. For example, depending upon the mode of device 50, a binding condition can be associated with an increase or decrease in channel conductance, a change in drain current or pinch-off voltage or threshold voltage, a change in transconductance, in channel width or depletion, and so on. One or more such characteristic changes may be measured by equipment 70 to provide a signal corresponding to attachment.

Preferably the variable power source 74 is adjusted, post-attachment, to restore whatever pre-attachment quiescent condition existed for device 50. Equipment 70 can measure the amount

of restoration bias required from power source 74 to provide an increased measure of the attachment. The compensating or restoration bias can be amplified, e.g., by amplifier 78, and the amplified signal coupled to one or more additional components 80. Component 80, in turn, could include a second device 200, coupled to perform a cascode amplification function upon the amplified restoration bias signal. If desired, the compensation bias signal from potential source 74 may be coupled to a feedback circuit 82 to automatically provide and maintain proper restoration bias (see also Figures 6A and 6B). The design of such feedback circuitry is well known to those skilled in the relevant art, and thus detailed information is not here presented.

With further reference to Figure 3A, as indicated by capacitor 82, the restorative bias from potential source 74 could in fact be an AC coupled pulse train, wherein one level corresponds to "pre-binding" and a second level of the pulse train corresponds to "post-binding". Because such pulse train signal is AC, capacitor 82 can decouple undesired effects of power source. This facilitates AC amplification of the detected signal, reducing susceptibility to problems associated with drift in DC amplifiers, power sources, etc. Further, capacitor 82, coupled in series with the bottom gate 62 as shown, determines the extent an applied AC compensating reverse bias from power source 74 affects device 50's channel conductance, or other operating characteristics. Voltage division, e.g., with a capacitor voltage divider, can also provide a "sensed" voltage gain, wherein a small fraction of the power source is used as a restorative potential, with the total power supply potential being measured to indicate the amount of gate charge attachment to be sensed.

The embodiment of Figure 3B is similar to what has been described except that while binding occurs in solution (as in Figure 3A), dry measurements are made. In practice, device 50 is exposed to the test solution 52 potentially containing the target substance 64. It is understood that in this embodiment, equipment 70 need not be present when or where device 50 is exposed to the target substance.

Materials needed for measurement enhancement and any confirmational testing need not exist at the time and place that testing occurred (only at the measurement site). This is advantageous, especially if the testing-binding site is in a remote location, or a location not amenable to storing enhancement materials under controlled conditions.

Applicant has discovered that such dry measurement advantageously eliminates the ion shielding problem so prevalent in the prior art. According to the present invention, removing the device 50 from an ionic solution 8 before testing facilitates elimination of the external reference electrode and attendant gate bias problems, drift and potential chemical contamination so prevalent in the prior art. In the present invention, attached surface charge is sensed directly.

Once a sensor 50 with bound target matter 64 is removed from an ionic test solution 52, the charges associated with the bound matter are no longer shielded by ions in the no longer present solution. As a result, a net charge is manifested at layer 60 generating an associated electric field across the underlying insulator layer 58. This charge and the attendant electric field attract oppositely charged free carrier charges from the semiconductor substrate 56 (or repel like polarity free carrier charges), resulting in opposite charges at or near the surface of the substrate 56. It is to be noted that the effects of contact potential at the sensor gate are generally different than the effects of bound charges.

Contact potential and charge attachment influences on a sensor, arising from target material attachment, may be separated by comparing the influence of target material attachment on the topgate region 66 of two sensor devices 50, 50' identical except for their insulator thickness 91. Separating the effects of these two phenomena occurs because while the attached charge generates an electric field across an insulator independent of insulator thickness, the contact potential induced electric field is inversely dependent upon insulator thickness. Thus, making two independent measurements of two unknowns permits identifying charge attachment effects, and contact potential effects upon sensor device 50.

Where the desired information is a direct measurement of target material concentration, binding rate of the target material to a receptor, dissociation rate, binding energy, etc., the test may be interrupted before steady state is achieved.

According to a preferred embodiment depicted in Figure 5, substrate 56 can include an on-chip pH sensor 100 and storage mechanism 102 for memorializing the test solution's pH value. The on-chip pH sensor 100 itself may be implemented as a device, according to the present invention, and may include the various enhancement techniques described herein.

Further, various confirmational devices, devices for measuring cofactors, and devices for measuring other chemicals of interest and for measuring chip receptor condition and integrity can be included on the same semiconductor chip. Preferably the sensor system further includes an on-chip mechanism 104 for measuring and storing the pre-attachment quiescent conditions of one or more devices (e.g., 50A, 50B, etc.) fabricated on the same substrate. For ease of illustration, Figure 5 does not depict the couplings between device 104 and the various sensors.

As depicted by Figure 5, the sensor system 106 can include measurement circuitry, e.g., 104, for each sensor device, and circuitry providing error alerts where, for example, receptor integrity, storage conditions, etc., are in question. Such on-chip circuitry can include a temperature sensor to monitor incubation conditions present during the chemical reaction of

interest (including attachment and dissociation), as well as a clock measuring reaction or dissociation process times. Thus, system 106 can measure physical parameters including reaction temperature, reaction time, solution pH, and ionic concentration.

In the generalized embodiment of Figure 5, note that it is not necessary that each sensor be identical, either in size, or in the density and/or number of receptor types 66, 66'. For example, sensor 50F includes no receptors and may be coated with an inert material, and may be used as a calibration sensor in that the "before" and "after" binding characteristics of sensor 50F should be the same. Confirmational testing excluding a non-specific reaction with device 50F's inert material could be achieved by providing another sensor whose outer film should also be non-reactive with the test solution contents. "Before" binding data from sensor 50F may be stored in device 104 for comparison to the sensor's "after" binding data. If the two sets of data do not agree, a user would know to suspect the integrity of data from sensors 50A-50E. For example, the array 106 of sensors may have been damaged at some time.

The embodiment of Figure 5 also promotes confirmatory testing not merely of the devices themselves, but of various target materials. For example, device 50B has a greater density of the same type of receptors than does device 50A. Therefore confirmation of binding the appropriate target material for receptors 66 would be indicated by a proportionally greater binding effect upon device 50B contrasted with device 50A.

Further, by providing some devices with only a first type of receptor (e.g., device 50A), and some devices with only a second type of receptor (e.g., device 50C), a differential analysis of a binding event may be made. For example, a target substance suitable for binding only with receptors 66 should produce a measurable change in device 50A, but not in device 50C. Further, a device such as device 50E may include both sensor types to provide still further confirmatory information as to the nature of what the binding substance is. Additional confirmational data may be acquired by re-exposure of a previously exposed test device to a different temperature, different pH, different chemicals, etc., providing known results for the target material. For example, disassociation rate of nucleic acid components with temperature, dissociation with pH change, charge change with pH change, charge sign change with pH change, disassociation as a function of different receptor binding sites, etc., provide useful information, especially as to whether what bound to the receptor of interest was in fact the desired target material.

Differential analysis could also be performed by exposing two identical sensors to a target material for two different time lengths, each shorter than the time known to be required for the target-receptor reaction to complete. Charge measurement for each device provides data for

target material reaction (or attachment) rate, and concentration in the test solution, data useful to provide species identification.

Whether measurements are conducted wet (as depicted in Figure 3A) or dry/quasi-dry (as depicted in Figure 3B), the bound charges result in an electric field that can modulate the conductance of the device semiconductor channel (if not neutralized by shielding, etc.). This modulation permits detection using suitable equipment 70, according to the present invention. While the description thus far has been with respect to a sensor 50 such as depicted in Figures 3A and 3B, according to the present invention, other semiconductor field effect sensors including the embodiment of Figure 4 (and devices functionally similar thereto) could also be used.

The present invention also facilitates obtaining sequence information of polymeric molecules such as DNA, RNA, glycoconjugates, polypeptides, and so forth. Another preferred sensor embodiment that is very well suited for detecting binding attachment in a sensor application is depicted in Figure 4. Device 50' is a so-called distributed channel bipolar device ("DCBD"), similar to what is disclosed in U.S. Patent No. 4,885,623 to James Holm-Kennedy, et. al. (1989). The DCBD structure of device 50' includes FET components gate oxide insulation layer 58', an appropriate moisture blocking layer 90', gate 60', drain 100, channel region 56', as well as bipolar components base 104 and emitter 106. While Figure 4 depicts a DCBD device with vertical architecture, alternatively, a DCBD device could be fabricated with lateral architecture, or a combination of vertical and lateral architecture. Similar to what was set forth above with reference to device 60 of Figures 3A and 3B, in sensor applications, the gate 60' supports an moisture blocking protective film 90' to whose outer surface primary receptors 66' are attached.

As described in detail in said U.S. Patent No. 4,885,623, depending upon mode of operation, the DCBD may be treated as an FET device with a bipolar source or drain that may be distributed. The DCBD may also be treated as a bipolar device with an FET emitter or collector that may be distributed, or as a gated bipolar device. The DCBD may be operated in enhanced or depletion mode. Further, vertical and lateral bipolar devices can be incorporated in a DCBD device. The FET portion of a DCBD device may incorporate a MESFET, JFET, or IGFET structure.

DCBD devices are especially well suited for sensor wet and dry testing, according to the present invention. DCBD devices exhibit high transconductance and current gain sensitivity to relatively small changes in gate voltage or charge, resulting from the binding of a target substance to the device. DCBD devices are also very sensitive to small changes in base current, under certain operating conditions. Further, techniques may be employed to enhance

various DCBD parameters in response to target binding. Such enhanceable parameters include bipolar gain, gate and base current dependent current gain, heterogeneous channel behavior, effective gate area and gate shape, and transconductance threshold voltage effect. Transconductance measurements with device 50' are especially advantageous because a null may be detected using alternating current ("AC") amplifiers, operating at high gain. Electrophoresis applications are also applicable to sensor devices such as the present invention.

With reference to Figures 6A and 6B, two embodiments of feedback for sensor reset and sensitivity enhancement measurement approaches are shown. In Figure 6A, sensor 50 is biased by a pulsed backgate bias supply 234, to reset or restore device 50 to its preattachment condition, while measurement system 230 monitors a device operating parameter sensitive to charged target attachment. For ease of illustration, parameter measurement system 230 is indicated generally, without a specific coupling 232 to the device to illustrate that the chosen parameter(s) may be varied, and can include current, voltage, transconductance, etc. The sensed parameter provides a feedback signal to feedback system 240 that, in response, adjusts the backgate operating voltage to restore the preattachment conditions.

In Figure 6A, the reset voltage from supply 234 is coupled to the input of a second device, FET 2, selected for high gain (e.g., a thin gate oxide device). In this cascode circuit, output current I_d 212 from sensor device 50 is amplified by FET 2, whose FET 2 output may be used to detect the target attachment event. Further, this output signal 212 may be processed with circuit 214 to provide additional information, and/or may be coupled to a feedback circuit 240 (shown in phantom) for restoring preattachment device 50 conditions. If desired, FET 2 may be a component in a circuit whose performance is affected by FET 2's change in channel resistance responding to attached charge at device 50. For example, if FET 2 affects the frequency of an oscillator 210, attachment at device 50 may be sensed by monitoring frequency change at oscillator 210.

In Figure 6B, a constant current is maintained in device 50, whereupon a certain voltage is seen across device backgate 270. Charge attaching to the topgate 272 of device 50 will induce a backgate voltage change, which change provides a measure of the attached charge. The current I_d may be AC pulsed, which allows AC amplification 237 without problems associated with DC amplifier drift.

In this example, the net charge upon device 50 from target 64 has been increased, including charge effects from the additional attachment of receptors to the "sandwich". Numerous other means of enhancing or amplifying the signal generated by target binding are available to those skilled in the relevant art. For example, as depicted in the right-hand portion of Figure 7B, a conglomerate may be used in the third sequence, wherein device 50 could be exposed to material having substantially more charge than target 64. For example, such material might

include a DNA chain (or fragment) 112 attached to a target substance 64X that will bind to the receptor 66X. In this fashion, a substantially larger net charge attaches to the field effect sensor device, resulting in a larger signal. It is understood that charge associated with receptor 66 and target 64 may be additive or subtractive, as may also be the case with material 66X, 64X and 112.

Figure 7C depicts the use of beads as an alternative (or additional) method of passively enhancing device sensitivity. Beads 114 may have a variety of shapes, are commercially available in sizes ranging from about 0.1μ to several μ, and are glass or polymer, e.g., materials affording many binding sites for a plurality of secondary receptors 66'. Receptors 66' have similar selective reactivity characteristics as the primary receptors 66, and may in fact be identical thereto. Beads, like conjugates, are commercially available and are frequently made from gold, biotin, ferritin, and the like, and may be made from other chemicals such as nucleic acid chains and antibody chains. In a second sequence, after target 64 has bound to primary receptor 66, the device 50 is exposed to a typical solution containing conjugated receptors 116.

With reference to Figures 8A-8C, the drain current (Ids) and drain-source voltage (Vds) for sensor 50 was measured after the application of various biolayers. For the data shown in these figures, incubation was 27°C with a 10mM MOPS buffer employed to maintain pH ≈ 7. With reference to Figure 8B, for example, the device was tested before and after application of a parylene film 90 (bar graphs a and b). After stabilizing the device in a MOPS buffer solution measurements were again taken (bar graph c). A receptor layer (receptor:IgG) was applied, and measurements taken (bar graph d), after which the device was exposed to a BSA blocking agent and measured (bar graph e). The utility of the BSA blocking layer is shown by bar graph f (Figure 8B), wherein the device was exposed to a non-specific antibody. As shown by bar graphs e and f, there was relatively little change in charge from non-specific bindings, which indicates BSA suppression of non-specific binding. Next the device was exposed to a desired target substance (antigen: Goat anti-IgG), and a change in charge (ΔQ) was noted (bar graph g). Finally, the device was reexposed to receptor material (IgG), which resulted in essentially the same charge change (ΔQ). It is noted from Figures 8B and 8C that different chemical exposure and reactions can result in charge attachment of different polarities, as indicated by the direction of the bar shift after exposure to subsequent chemical exposure.

The data for bar graph a in Figure 8C depicts a denatured device 50. After denaturing, the device was exposed to a buffered MOPS solution (bar graph b), which altered the effective charge on the device. Thereafter in a suitable incubation environment, receptors were attached (bar graph c), which in this case altered the gate attached charge. Exposure to a BSA

environment (bar graph d) slightly changed the charge, and exposure to the target material (bar graph f) resulted in a desired perceptible charge change ΔQ.

Automated testing is readily implemented using the invention, which can include on-chip testing circuitry (see Figure 5). Dry testing particularly promotes automated procedures, where the bound sensors may be automatically delivered to a test fixture (e.g., a test probe), where test information is read and the test results stored for automatic computer processing.

While the present invention has been described with reference to a few specific embodiments, the description is illustrative of the invention and is not to be construed as limiting the invention. Various modifications may occur to those skilled in the art without departing from the true spirit and scope of the invention as defined by the appended claims.

WHAT IS CLAIMED IS:

1. A semiconductor device (50) for sensing without a reference electrode the presence of a desired particle (64) in a first solution (52), the device comprising:

a substrate (51);

a topgate region (60), including a chemically selective material (66), producing a desired first electrical signal upon attachment of said desired particle (64), said topgate region (60) overlying said substrate (51);

a field effect channel region (56) in said substrate at least partially underlying said topgate region;

a backgate region (62) adjoining said field effect channel region (56);

wherein said field effect channel region (56) is in field communication with said topgate region (60) and with said backgate region (62), said channel (56) being modulatable in conductance by an electrical signal from said topgate region (60) and/or from said backgate region (62);

wherein said desired first electrical signal modulates said channel's (56) preattachment conductance, providing an output signal from said device (50) indicating attachment.

2. The device of claim 1, wherein said field effect channel region (56) has channel characteristics similar to at least a chosen one of the group consisting of a junction field FET, a non-metalized MOSFET, a non-metalized MISFET, an ISFET, an inversion FET, a DCBD, a heterojunction FET, a transistor, and a capacitor.

3. The device of claim 1, further including means, coupled to said backgate, for enhancing an output signal indicating attachment.

4. The device of claim 3, wherein said means includes first means for monitoring a parameter of said device, and bias means for adjusting a bias at said backgate after attachment to restore said parameter to a preattachment state, wherein the bias adjustment provides a measure of attachment.

5. The device of claim 1, wherein said backgate comprises a semiconductor junction whose dopant concentrations and/or doping profiles are selected to cause a desired small fraction of an applied backgate bias voltage to modulate said channel conductance.

6. A system for sensing the presence of a desired particle (64) in a first environment (52), the system comprising:

a substrate (51);

at least one semiconductor sensor (50), on said substrate (51);

first electrical means (100), on said substrate (51), for measuring at least one parameter of said first environment (52);

second electrical means (102), on said substrate (51), coupled to an output of said first electrical means (100) for memorializing at least one parameter of said first environment (52).

7. A method for detecting and measuring the presence of a desired particle in a first solution by its attachment to a field effect device, the method comprising:

exposing the field effect device to said first solution, wherein said field effect device includes a surface adapted to bindingly attach to said desired particle, said attachment altering at least one parameter of said device from a preattachment state;

at least quasi-drying said device; and

measuring, in a second environment, said at least one parameter to determine whether attachment of said desired particle occurred.

8. The method of claim 7, wherein said second environment is selected from the group consisting of air, and a second solution substantially different in pH, temperature and/or chemical composition from said first solution.

9. The method of claim 7, further including the step of providing means for modify charge associated with said attached target.

10. The method of claim 7, wherein a chosen region of said surface of said device is receptive to said attachment, said chosen region resulting from a chosen step selected from the group consisting of (a) embedding a shielding layer (214) in said device (50) during device fabrication, which layer shields said device save for a region underlying said chosen region, and (b) forming a pattern mask of inert material (210) on all of said surface save for said chosen region thereof.

AMENDED CLAIMS

[received by the International Bureau on 12 March 1993 (12.03.93); original claims 1-5 amended; other claims unchanged (2 pages)]

1. A semiconductor device (50) for sensing without a reference electrode the presence of a desired particle (64) in a first solution (52), the device comprising:

a single substrate (51);

a topgate region (60), including a chemically selective material (66), producing a desired first electrical signal upon attachment of said desired particle (64), said topgate region (60) overlying said substrate (51);

a field effect region (56) in said substrate at least partially underlying said topgate region;

a backgate region (62) adjoining said field effect region (56); and

means (50', or 62 or 74 or 82 or 200 or 212 or 230 or 234 or 240 or 270) for enhancing measurement sensitivity of said semiconductor device (50) to attachment of said desired particle (64);

wherein said field effect region (56) is in field communication with said topgate region (60) and with said backgate region (62), said field effect region (56) being modulatable in conductance by an electrical signal from said topgate region (60) and/or from said backgate region (62);

wherein said desired first electrical signal modulates said field effect region's (56) preattachment conductance, providing an output signal from said device (50) indicating attachment.

2. The device of claim 1, wherein said field effect region (56) is a field effect channel region having channel characteristics similar to at least a chosen one of the group consisting of a junction field FET, a non-metalized MOSFET, a non-metalized MISFET, an ISFET, an inversion FET, a DCBD, a heterojunction FET, a transistor, and a capacitor.

3. The device of claim 1, wherein said means for enhancing measurement sensitivity includes electrical means, coupled to said backgate, for enhancing an output signal indicating attachment.

4. The device of claim 3, wherein said electrical means includes first means for monitoring a parameter of said device, and bias means for adjusting a bias at said backgate after attachment to restore said parameter to a preattachment state, wherein the bias adjustment provides a measure of attachment.

5. The device of claim 1, wherein said backgate comprises a semiconductor junction whose dopant concentrations and/or doping profiles are selected to cause a desired small fraction of an applied backgate bias voltage to modulate said field effect region conductance.

6. A system for sensing the presence of a desired particle (64) in a first environment (52), the system comprising:

a substrate (51);

at least one semiconductor sensor (50), on said substrate (51);

first electrical means (100), on said substrate (51), for measuring at least one parameter of said first environment (52);

second electrical means (102), on said substrate (51), coupled to an output of said first electrical means (100) for memorializing at least one parameter of said first environment (52).

7. A method for detecting and measuring the presence of a desired particle in a first solution by its attachment to a field effect device, the method comprising:

exposing the field effect device to said first solution, wherein said field effect device includes a surface adapted to bindingly attach to said desired particle, said attachment altering at least one parameter of said device from a preattachment state;

at least quasi-drying said device; and

measuring, in a second environment, said at least one parameter to determine whether attachment of said desired particle occurred.

8. The method of claim 7, wherein said second environment is selected from the group consisting of air, and a second solution substantially different in pH, temperature and/or chemical composition from said first solution.

9. The method of claim 7, further including the step of providing means for modify charge associated with said attached target.

10. The method of claim 7, wherein a chosen region of said surface of said device is receptive to said attachment, said chosen region resulting from a chosen step selected from the group consisting of (a) embedding a shielding layer (214) in said device (50) during device fabrication, which layer shields said device save for a region underlying said chosen region, and (b) forming a pattern mask of inert material (210) on all of said surface save for said chosen region thereof.

STATEMENT UNDER ARTICLE 19

Sakai, et al., U.S. Patent no. 4,961,833 was cited against originally filed claims 1-6, while Guckel, U.S. Patent no. 4,180,771 was cited against originally filed claims 1-12.

Applicant has amended claims 1-5. Provided herewith are substitute pages 22 and 23 containing claims 1-12, wherein claims 1-5 have been amended as above.

Amended claim 1 recites that the claimed device comprises a single substrate. This limitation is supported by any of Figures 2A, 2B, 3A, 3B, 4, 5, 6A, 6B. By contrast, Sakai, et al., U.S. Patent no. 4,961,833 discloses a device having first and second substrates (12, 14), separated by an oxide layer (13). See Sakai, et al., Figure 1. Amended claim 1 refers to region (56) as a field effect region, which it is, rather than as a channel, which it may be. See for example, the Specification, page 13, line 27.

Claim 1 has also been amended to require that the device include means for enhancing measurement sensitivity. This limitation is supported, inter alia, by Figures 2A, 2B, 3A, 3B, 4, 5, 6A and 6B. See also the accompanying text in the Specification at pages 11-13, 15, 16, and 19.

Claims 2-5 have been amended to conform to the nomenclature in amended claim 1.

<u>The Sakai, et al. Reference Distinguished</u>:
Sakai, et al. was cited against originally filed claims 1-6. Sakai, et al. discloses a structure wherein every embodiment has two substrates separated by an oxide layer. As noted, the presently claimed invention set forth in claims 1-5 requires but a single substrate. Further, the means for enhancing sensitivity now set forth in amended claim 1 is neither disclosed nor suggested in Sakai, et al.

Claim 6 requires, inter alia, first and second electrical means (100, 102), which means are neither disclosed nor suggested in Sakai, et al.

Applicant therefore submits that the invention set forth in claims 1-6 is neither anticipated by nor rendered obvious by Sakai, et al.

The Guckel Reference Distinguished:

Guckel was cited against originally filed claims 1-12. Amended claim 1 requires, inter alia, means for enhancing measurement sensitivity. Applicant submits that such means are neither disclosed nor suggested by Guckel. Further, the preamble to claim 1 recites that the claimed device is used for sensing without a reference electrode. Guckel's device includes such a reference electrode (see Guckel Figure 1, element 24), although Guckel appears to suggest that the reference electrode need not be used at all times.

Applicant submits that amended claim 1 is neither disclosed nor suggested by Guckel. Similarly, dependent claims 2-5 recite elements not disclosed or suggested by Guckel.

Claim 6 recites a system that includes, inter alia, first and second electrical means (100, 102), which means are neither disclosed nor suggested by Guckel, or a combination of Guckel and known prior art.

Method claims 7-10 include, inter alia, the step of at least quasi-drying a field effect device after attachment has occurred and before measurements are made. Further, claims 7-10 require that measurement occur in a second environment. Claim 8, for example, states that the second environment may be air. Claim 9 requires the further method step of providing means for modifying the charge associated with an attaching target.

Applicant submits that Guckel alone or in combination with known prior art does not disclose or suggest the method set forth in claims 7-10.

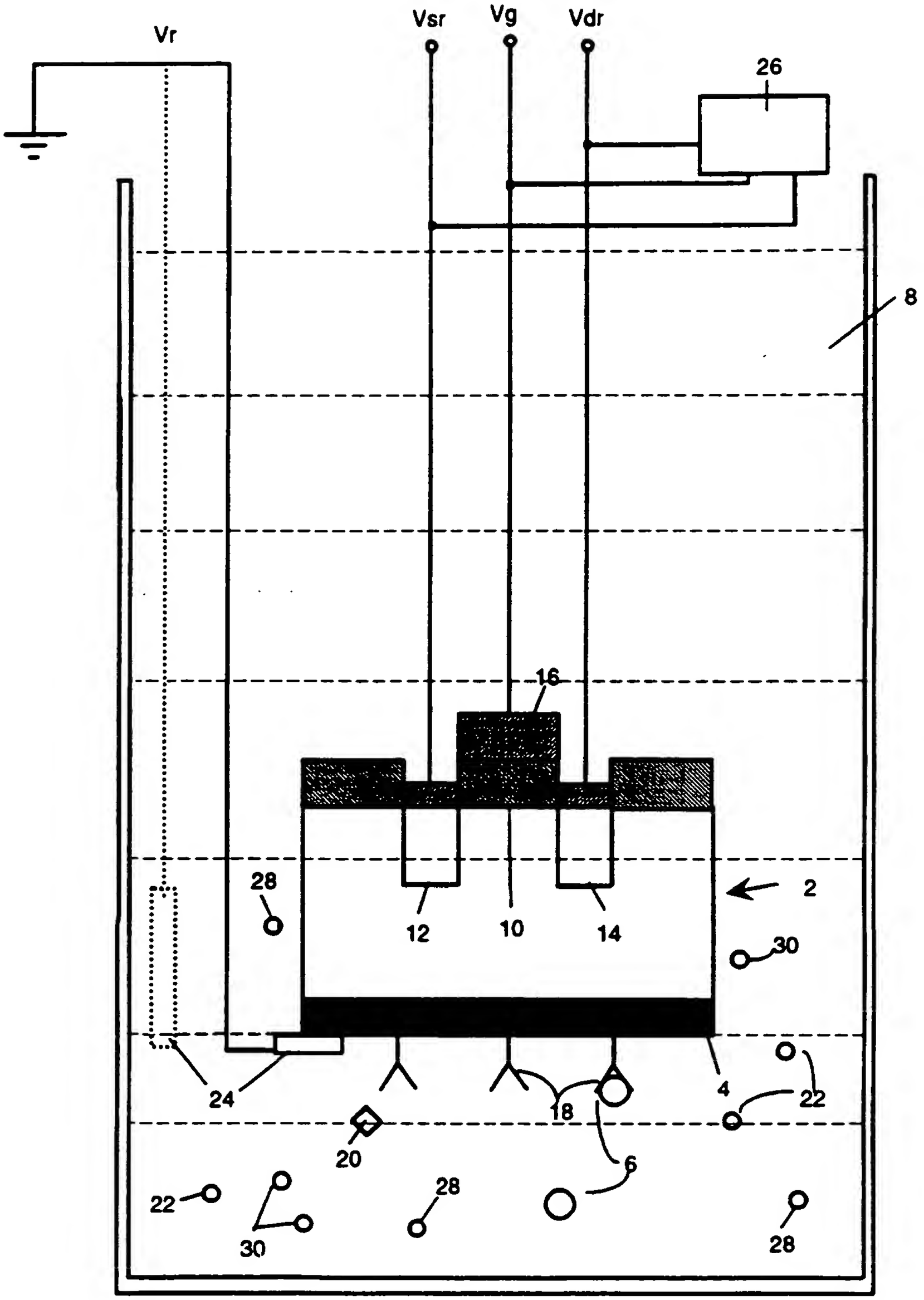

FIGURE 1

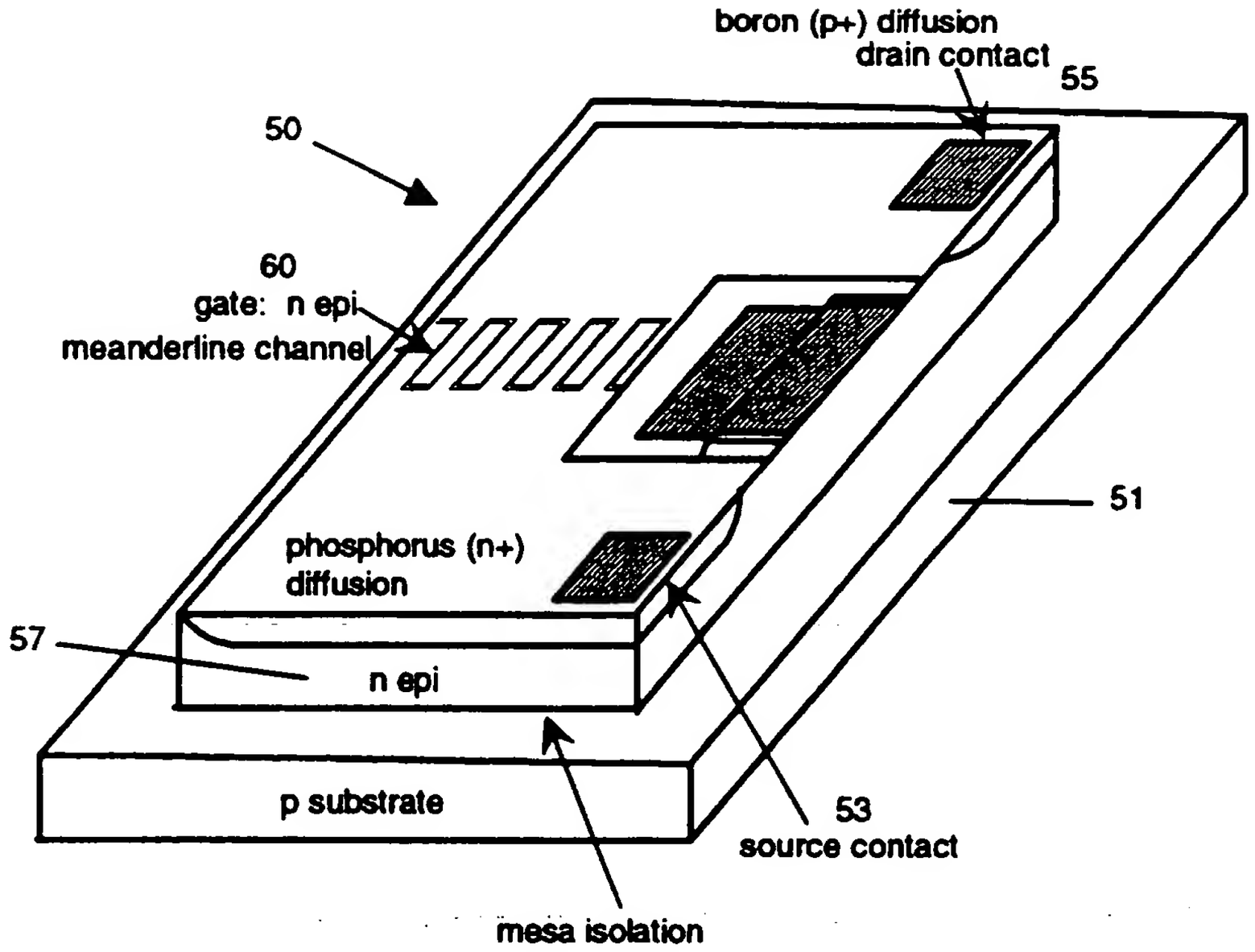

FIGURE 2A

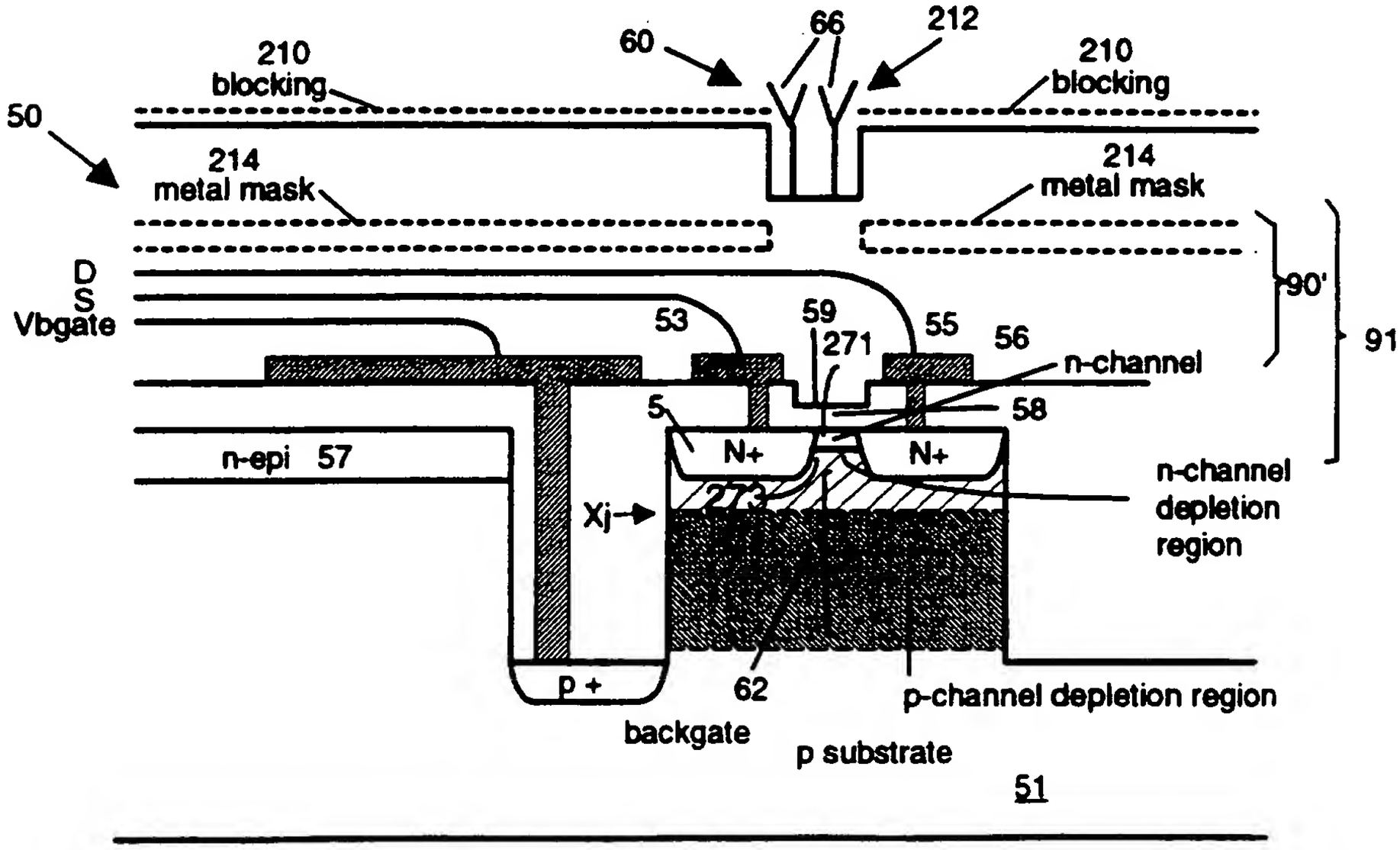

FIGURE 2B

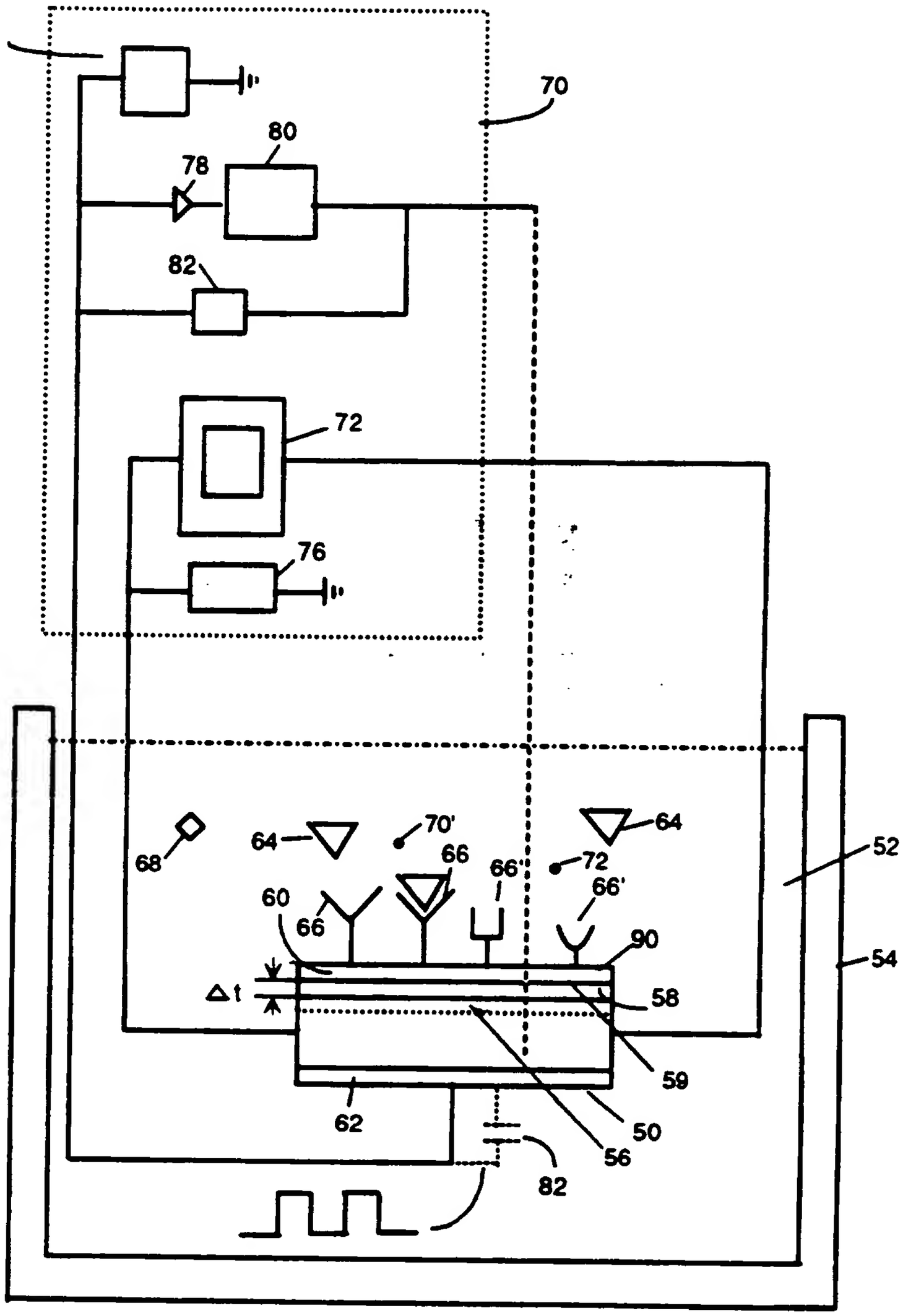
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FIGURE 3A

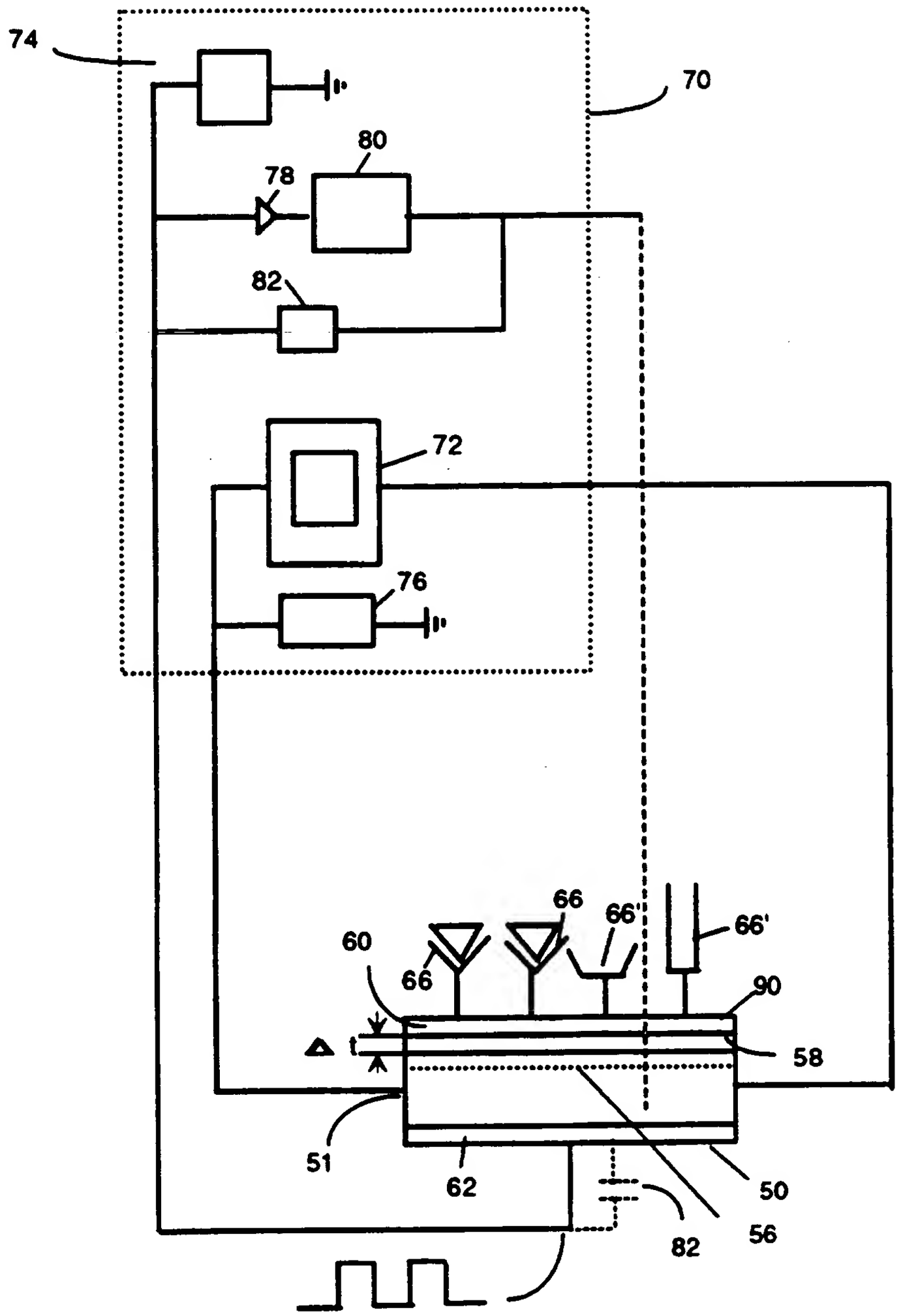

FIGURE 3B

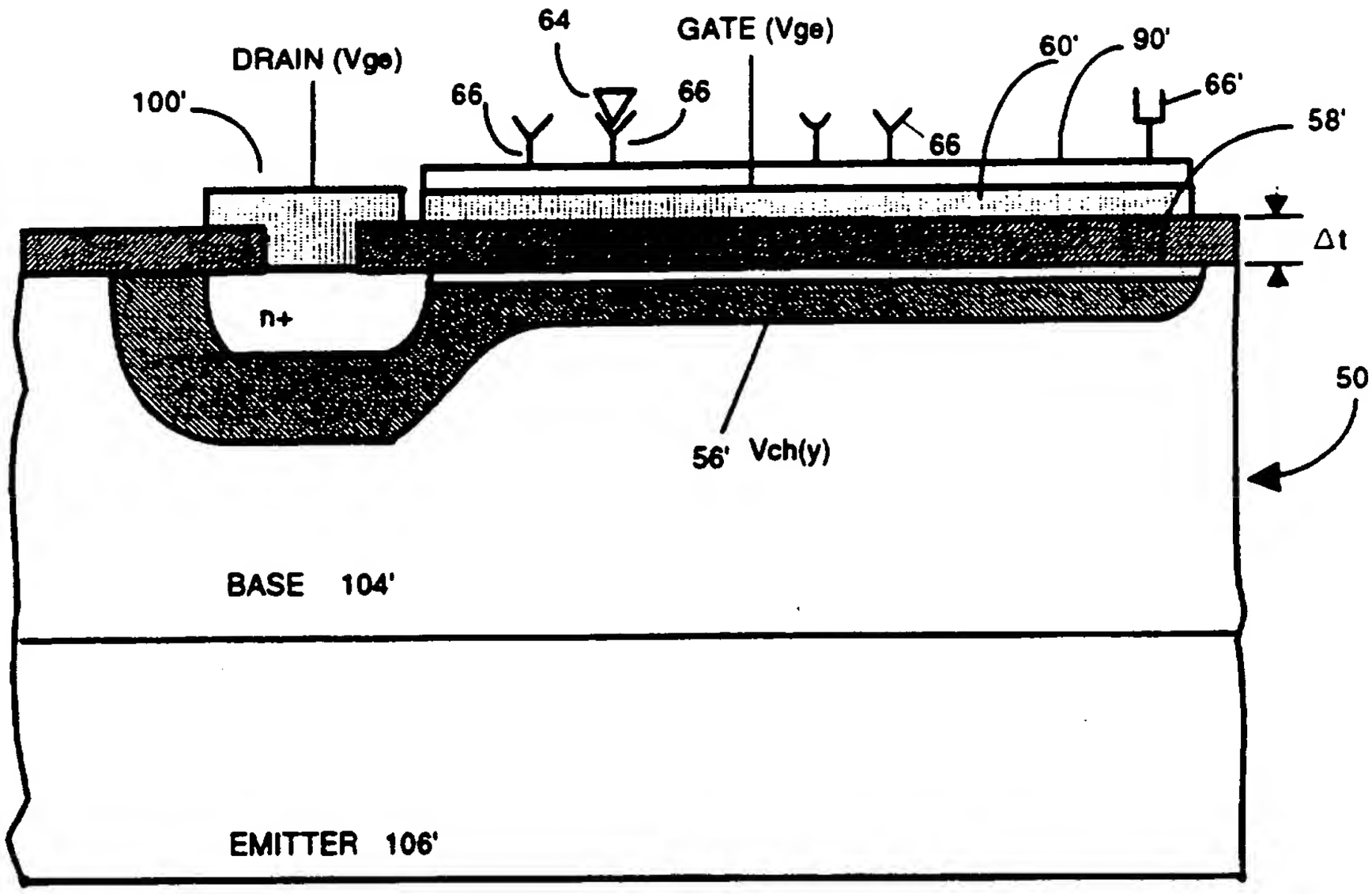

FIGURE 4

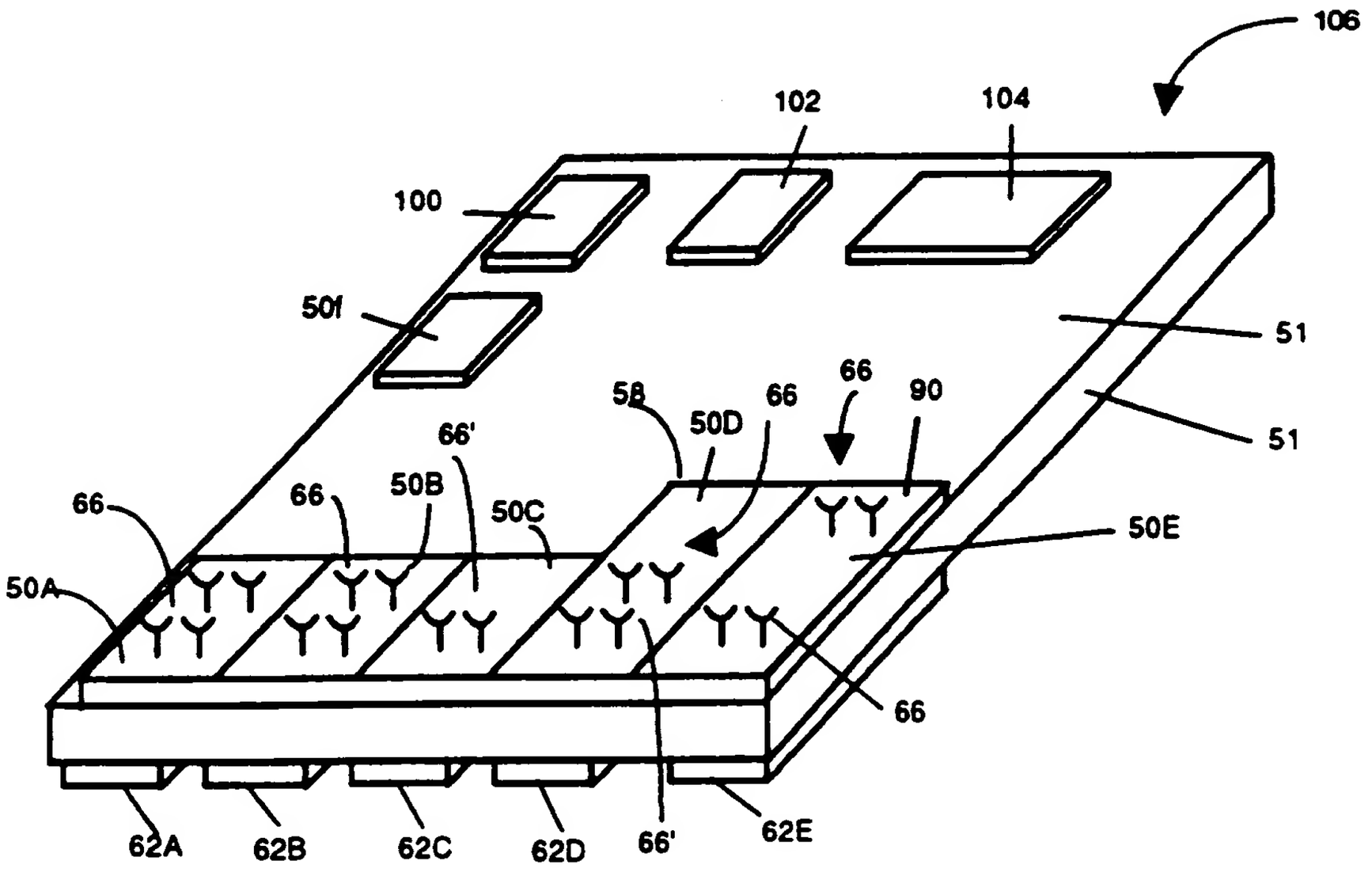

FIGURE 5

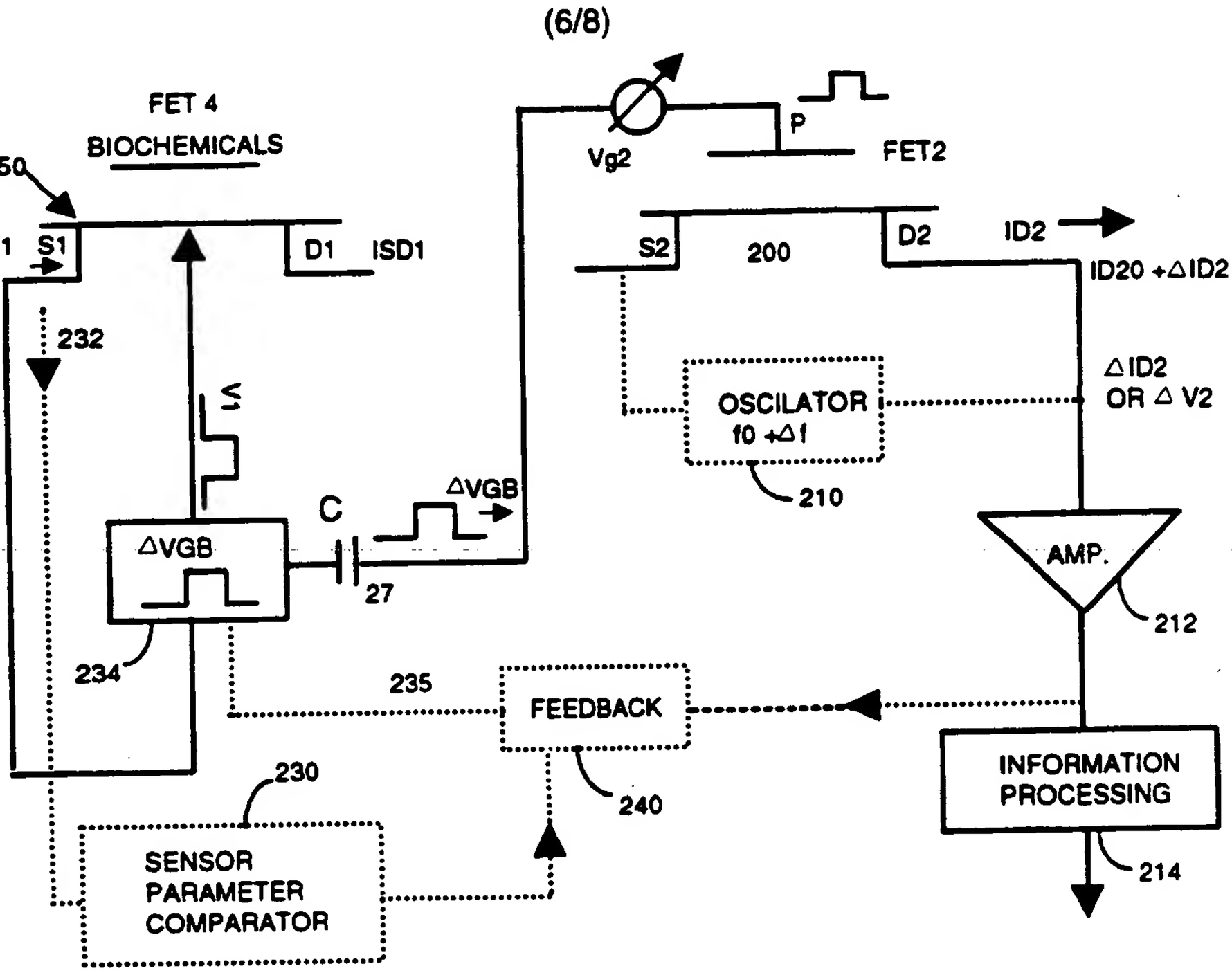

FIGURE 6A

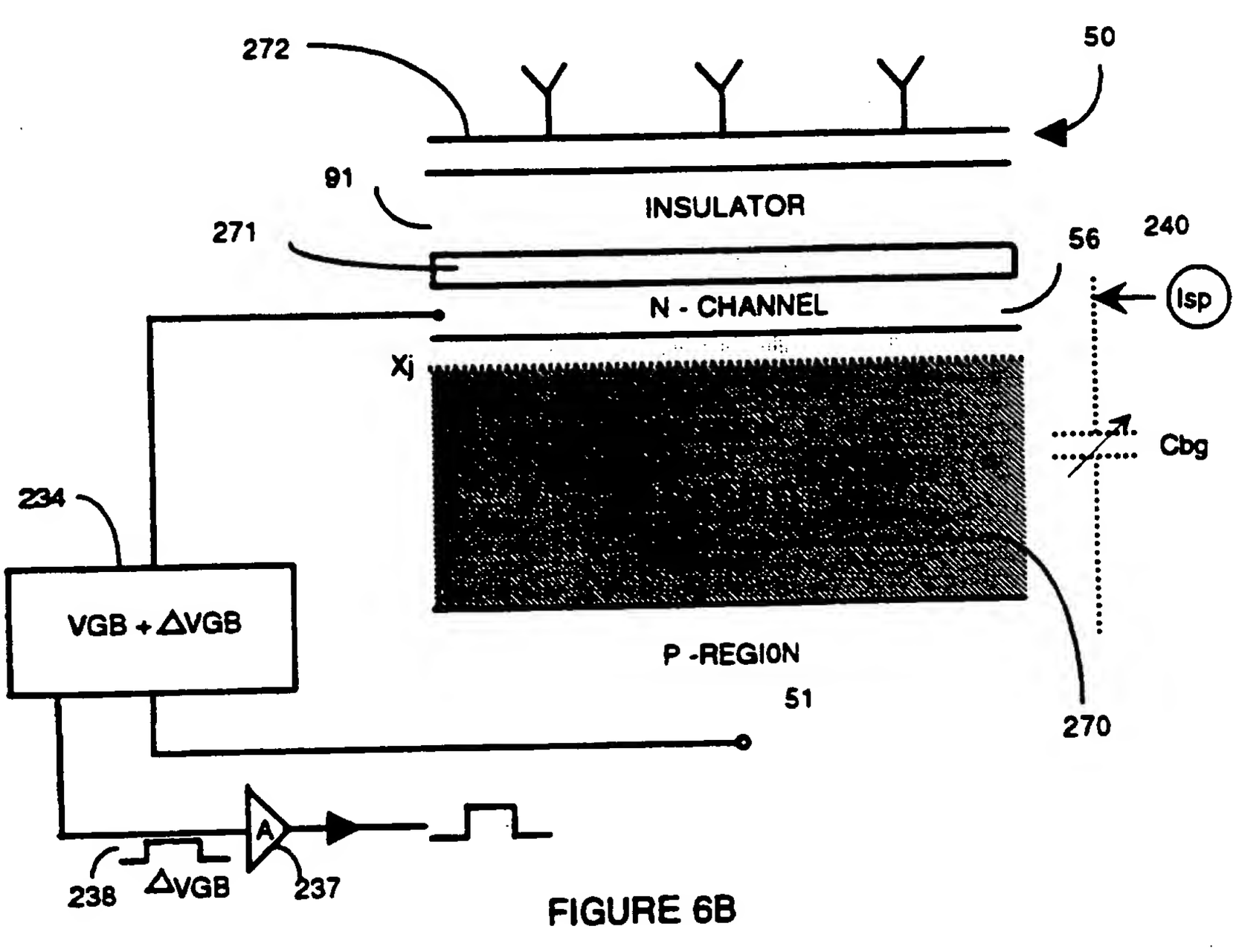

FIGURE 6B

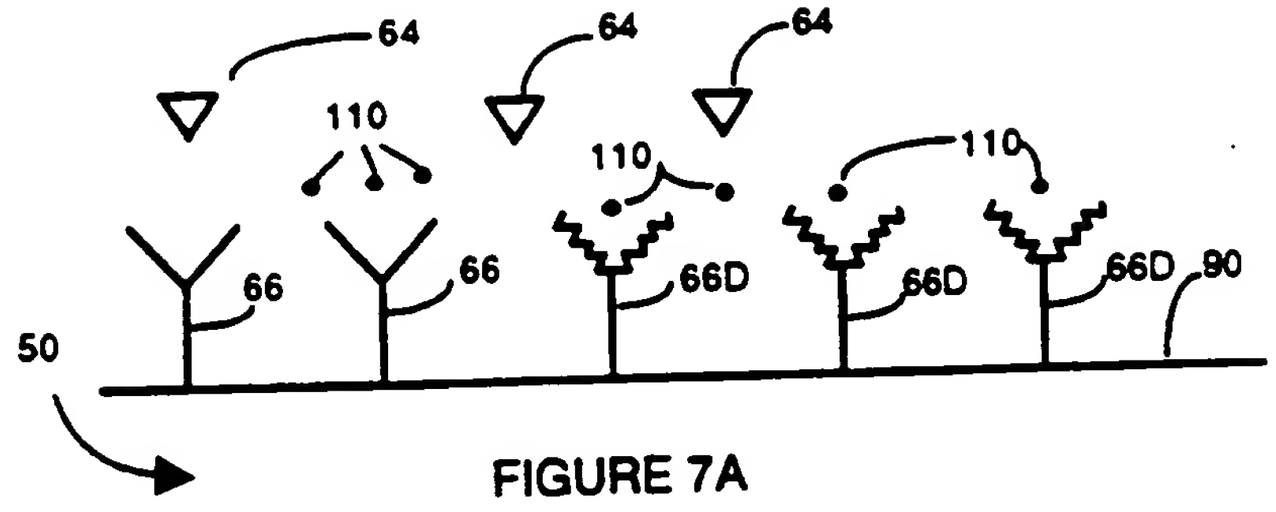

FIGURE 7A

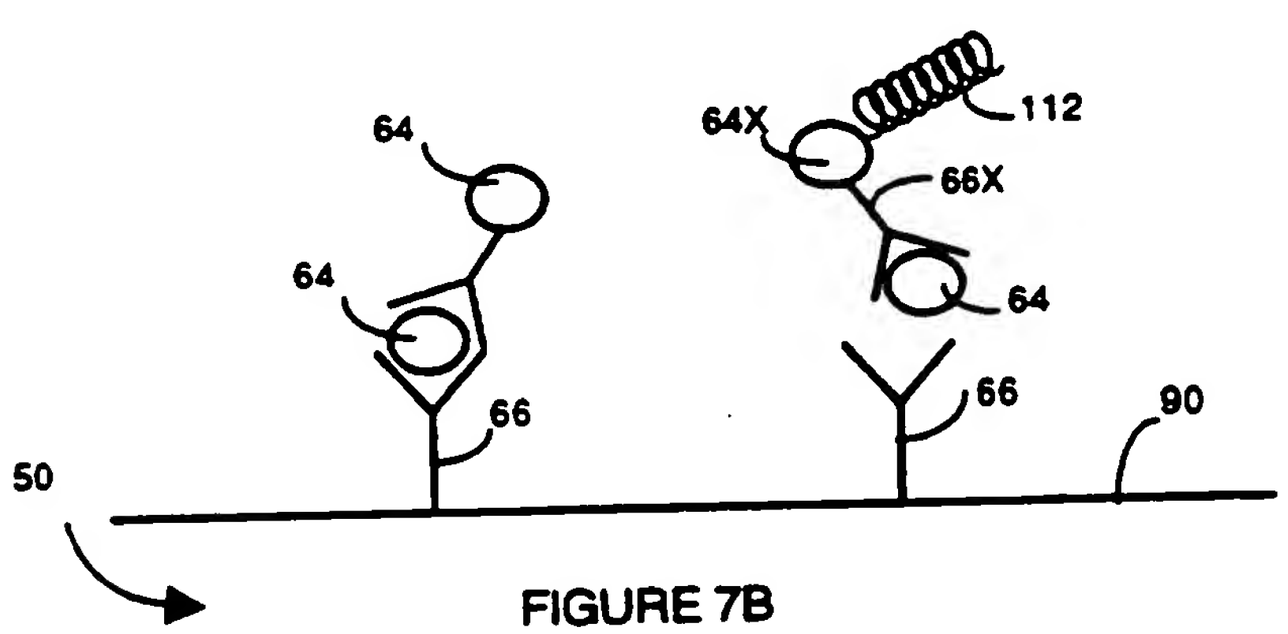

FIGURE 7B

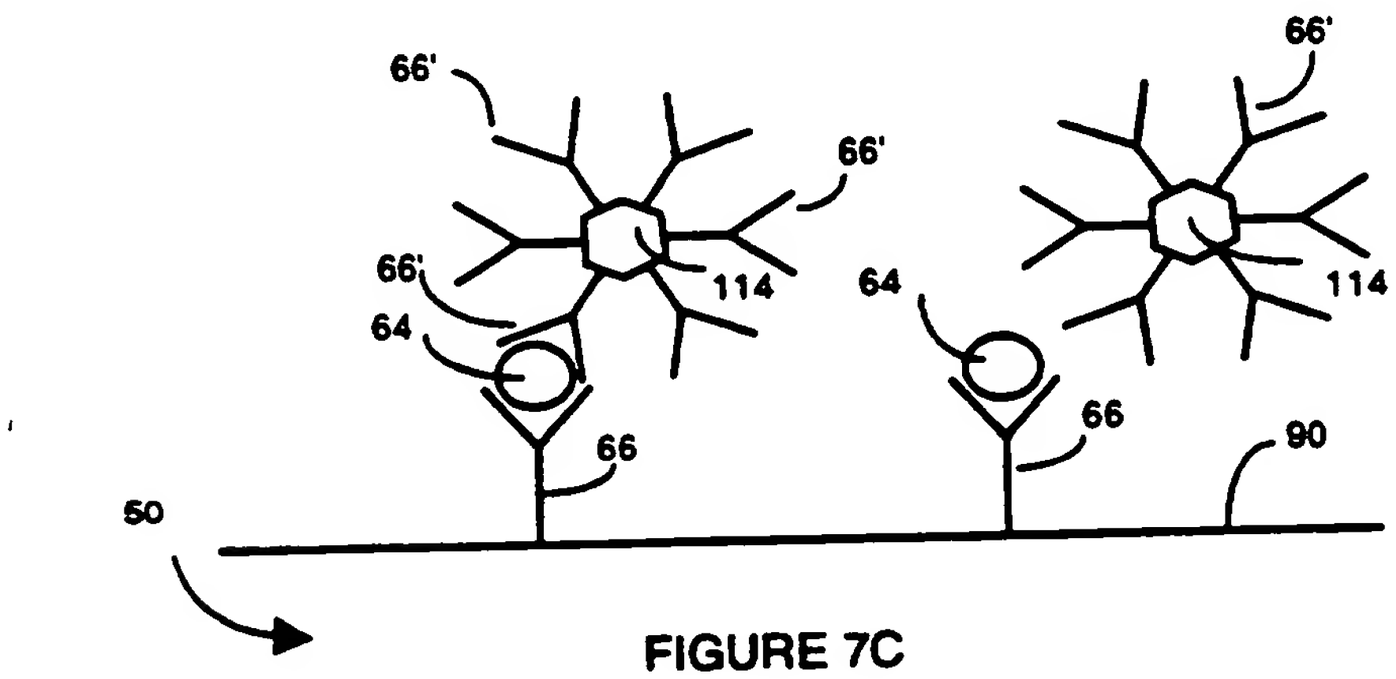

FIGURE 7C

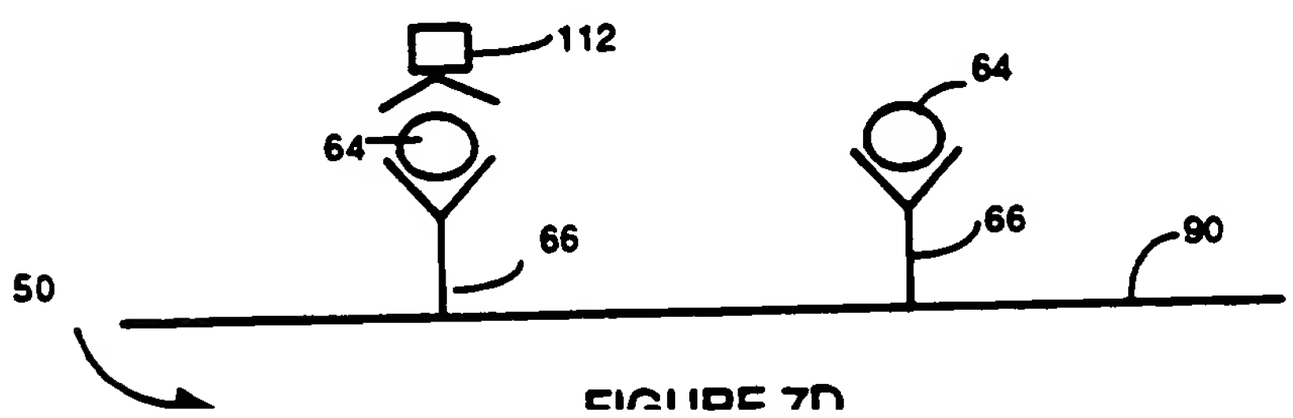

FIGURE 7D

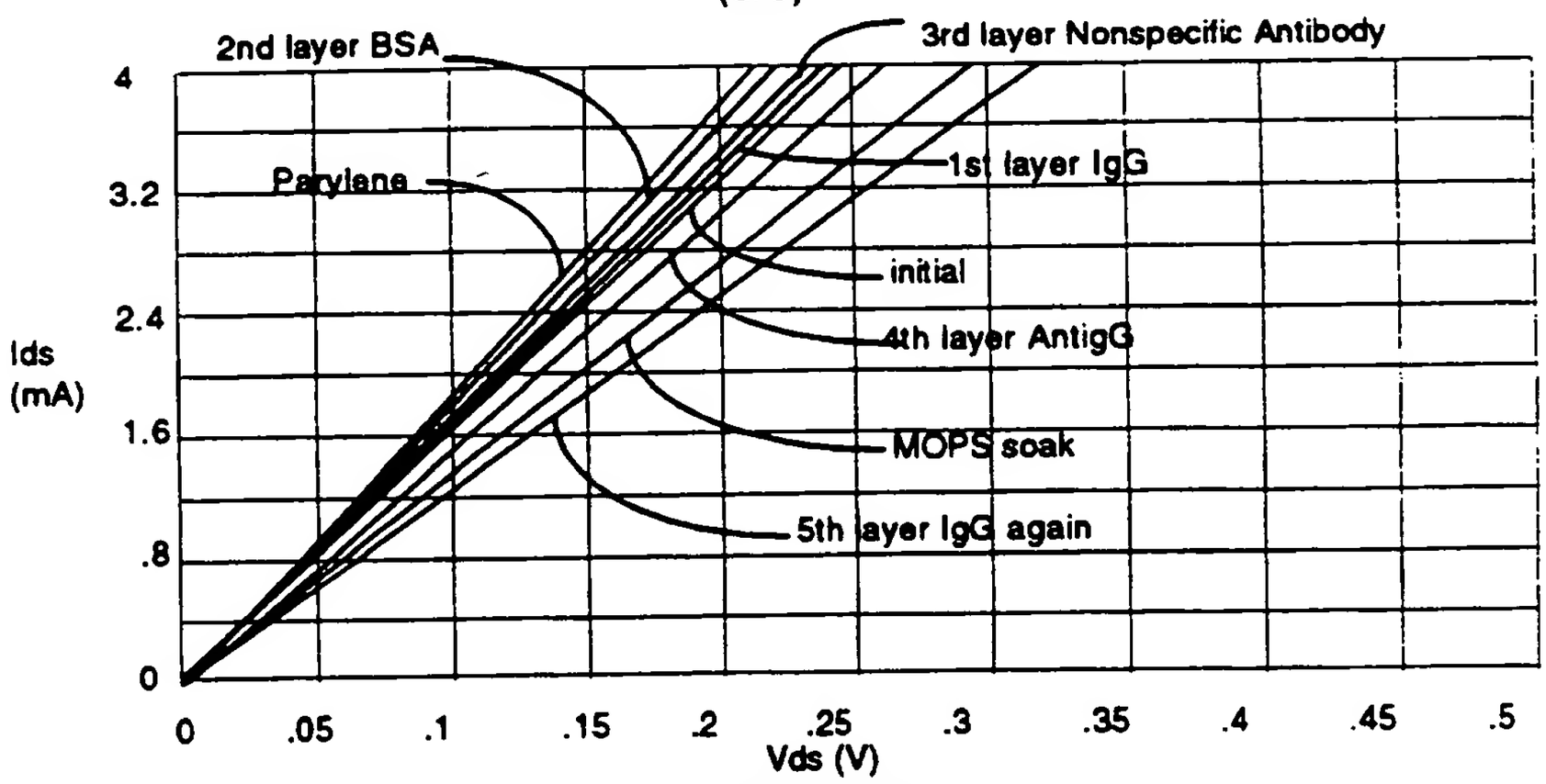

FIGURE 8A

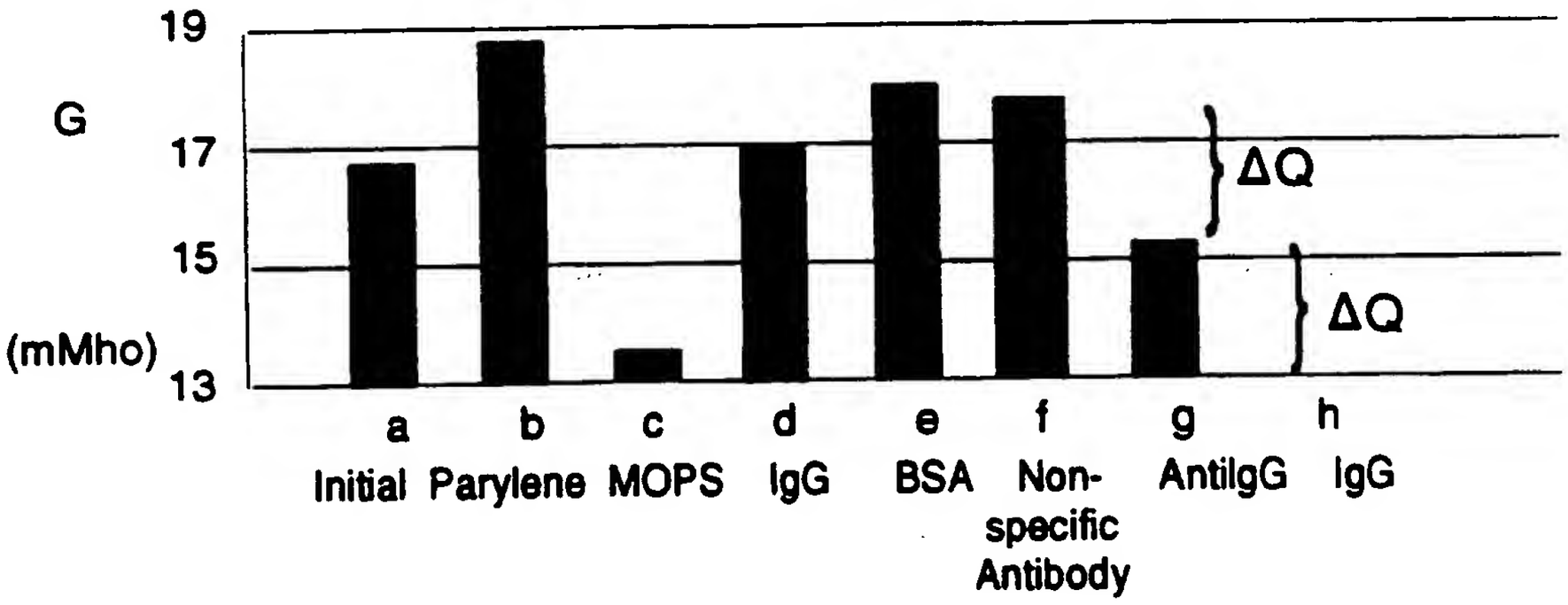

FIGURE 8B

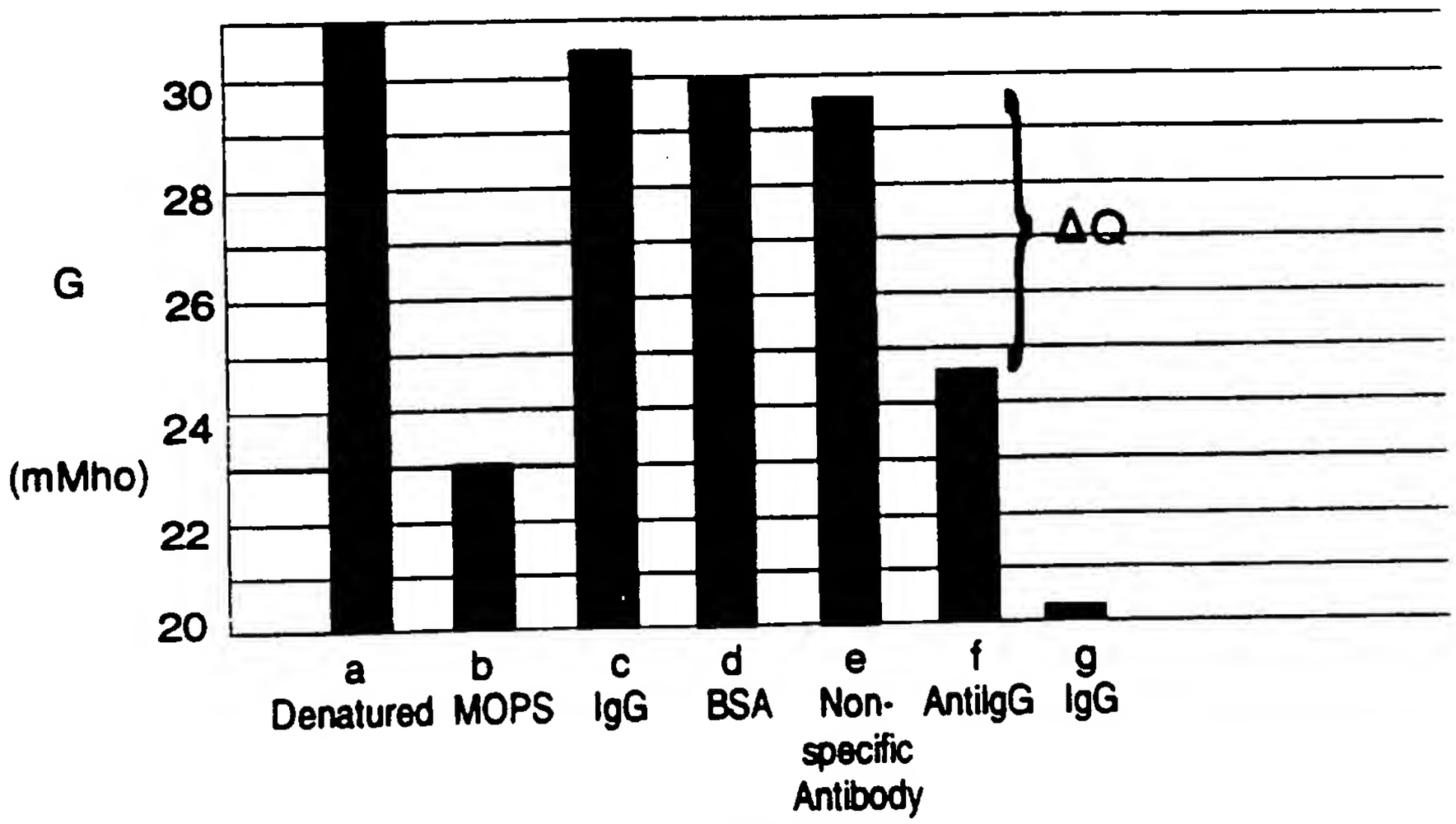

FIGURE 8C

INTERNATIONAL SEARCH REPORT

PCT/US92/08940

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :G01N 27/26; H01L 31/00
US CL :204/403.416.153.12; 257/253
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 204/403.416.153.12; 257/253

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
Y	US, A, 4,961,833 (SAKAI ET AL) 09 October 1990 (See column 3, lines 65-68 and column 4, lines 1-32)	1-6
Y	US, A, 4,180,771 (GUCKEL) 25 DECEMBER 1979, (See Abstract, column 3, lines 51-68 column 4, lines 27-68 and column 5, lines 1-2	1-10

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 NOVEMBER 1992	12 JAN 1993
Name and mailing address of the ISA/ US Commissioner of Patents and Trademarks	Authorized officer